

**AN INVESTIGATION OF THE
PSYCHOPHARMACOLOGY OF TIMING
BEHAVIOUR IN THE RAT**

by

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Abstract

Interval timing behaviour refers to the ability of animals and humans to adapt their behaviour to temporal regularities in their environments. Two important classes of interval timing behaviour are *temporal discrimination* (discriminating between the durations of external events) and *temporal differentiation* (behavioural adaptation during an ongoing interval). It has been known for many years that drugs that affect central dopaminergic function can alter both forms of timing behaviour. More recently, evidence has been accumulated which shows that manipulation of central 5-hydroxytryptaminergic (5-HTergic) function can also influence interval timing behaviour. The experiments described in this thesis examined the effects of drugs acting at some subtypes of 5-HT receptors on temporal discrimination and temporal differentiation in the rat.

Chapter 1 contains a review of the relevant literature. First, the anatomy, biochemistry and receptor pharmacology of the 5-HTergic system is outlined, and a selective review of the role of 5-HT in some behaviours relevant to this project is presented. This is followed by an overview of the behavioural methodology that has been used to study timing behaviour in animals, and an account of the major theories of timing behaviour. Finally, the behavioural pharmacology of timing behaviour is reviewed.

Chapters 2-7 describe a series of experiments examining the effects of drugs acting at 5-HT_{1A}, 5-HT_{2A/2C}, and 5-HT₃ receptors on temporal discrimination and temporal differentiation.

Experiment 1 examined the effect of the 5-HT₃ receptor agonist *m*-chlorophenylbiguanide (*m*-CPBG) and the non-selective agonist quipazine on

temporal discrimination performance in the discrete-trials psychophysical procedure. Quipazine produced a dose-dependent disruption of temporal discrimination, consisting of a rightward displacement and flattening of the fitted psychometric function, reflected in a significant increase in the values of the indifference point T_{50} and the Weber fraction. *m*-CPBG had no significant effect on either T_{50} or the Weber fraction. The effects of quipazine were completely abolished by the 5-HT_{2A} receptor antagonist ketanserin, but not by the 5-HT₃ receptor antagonist toparyl 3,5-dichlorobenzoate (MDL-72222), indicating that the effect of quipazine was mediated by 5-HT_{2A}, and not 5-HT₃ receptors.

In experiment 2, the effects of quipazine and *m*-CPBG were examined on temporal differentiation performance in the free-operant psychophysical procedure. Quipazine dose-dependently displaced the psychometric function to the left, reducing the value of T_{50} , and significantly increased the Weber fraction. *m*-CPBG had no effect on the parameters of the function. The effects of quipazine were reversed by co-administration of ketanserin, but not by co-administration of MDL-72222. These results suggest that while 5-HT_{2A} receptor stimulation has a robust influence on temporal differentiation, 5-HT₃ receptor stimulation does not.

Experiment 3 further examined the effect of 5-HT_{2A} receptor stimulation on temporal discrimination. The 5-HT_{2A/2C} receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) increased the Weber fraction and tended to increase T_{50} . Ketanserin and the highly selective 5-HT_{2A} receptor antagonist (±)2,3-dimethoxyphenyl-1-(2-(4-piperidine)-methanol) (MDL-100907) fully

antagonized the effects of DOI. The results indicate that DOI disrupts temporal discrimination via stimulation of 5-HT_{2A} receptors.

Experiment 4 examined whether intra-striatal injection of DOI would affect temporal discrimination, and whether the effect of systemically administered DOI on temporal discrimination would be blocked either by MDL-100907 or by 8-(5-(2,4-dimethoxy-5-(trifluoromethylphenyl-sulphonamido)phenyl-5-oxopentyl)-1,3,8-triazaspiro(4.5)decane-2,4-dione RS-102221: a selective 5-HT_{2C} receptor antagonist), administered directly into the dorsal striatum. Intra-striatal injection of DOI did not affect temporal discrimination. Systemically administered DOI disrupted temporal discrimination; this effect was not attenuated by intra-striatal injection of MDL-100907 or RS102221, suggesting that the 5-HT₂ receptors that mediate DOI's effect on temporal discrimination are not located in the dorsal striatum.

Experiments 5 and 6 examined the effects of intra-striatally administered DOI, MDL-100907 and RS-102221 on temporal differentiation. In experiment 5, systemic injection of DOI significantly reduced T_{50} . This effect was antagonized by systemically administered MDL-100907. In experiment 6, intra-striatally administered DOI had no significant effect on T_{50} or the Weber fraction. Intra-striatal injections of MDL-100907 and RS-102221 did not alter temporal differentiation, and failed to reverse the effects of systemically administered DOI. The results suggest that the 5-HT₂ receptor population responsible for DOI's effect on temporal differentiation is not located in the dorsal striatum.

Experiment 7 examined the effect of a 5-HT_{1A} and a 5-HT_{2A} receptor agonist on another widely-used temporal differentiation schedule, the fixed-

interval peak procedure. The 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and the 5-HT_{2A/2C} receptor agonist DOI had similar effects on performance: both agonists displaced the peak function to the left and reduced the peak time, t_{peak} . The effect of 8-OH-DPAT was antagonized by the selective 5-HT_{1A} receptor antagonist *N*-[2-(4-[2-methoxyphenyl]-1-piperazinyl)ethyl]-*N*-2-pyridinylcyclohexane-carboxamide (WAY-100635), and the effect of DOI by the 5-HT_{2A} receptor antagonist ketanserin, respectively. These results, taken together with previous findings with the free-operant psychophysical procedure, suggest that 5-HT_{1A} and 5-HT_{2A} receptors mediate similar effects on temporal differentiation.

The final chapter (Chapter 8) summarizes the findings from the project, and discusses their implications for the putative role of 5-HT in interval timing and for current theoretical accounts of timing. It is argued that current models of timing behaviour that assume the existence of a unitary ‘pacemaker-driven’ internal clock may have difficulty accommodating the finding that the same drug can have qualitatively different effects on temporal discrimination and temporal differentiation. Some possible directions for future research in this area are also discussed.

DECLARATION

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification in the University of Nottingham or any other university or institute of learning.

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PUBLICATIONS ARISING FROM THE PROJECT

Full-length peer-reviewed publications

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- BODY, S., ASGARI, K., RICKARD, J.F., ZHANG, Z., FONE, K.C.F., BRADSHAW, C.M. & SZABADI, E. (2005): Effects of quipazine and *m*-chlorophenylbiguanide (*m*-CPBG) on temporal differentiation: evidence for the involvement of 5-HT_{2A} but not 5-HT₃ receptors in interval timing behaviour. *Psychopharmacology*, in press.
- ASGARI, K., BODY, S., FONE, K.C.F., BRADSHAW, C.M. & SZABADI, E. (2006): Effects of 5-HT_{1A} and 5-HT_{2A} receptor stimulation on temporal differentiation performance in the fixed-interval peak procedure. *Behavioural Processes*, in press.
- BODY, S., ASGARI, K., CHEUNG, T.H.C., BEZZINA, G., GLENNON, J.C., BRADSHAW, C.M. & SZABADI, E. (2006): Evidence that the effect of 5-HT₂ receptor stimulation on temporal differentiation is not mediated by receptors in the dorsal striatum. *Behavioural Processes*, in press
- ASGARI, K., BODY, S., BAK, V.K., ZHANG, Z., RICKARD, J.F., GLENNON, J.C., FONE, K.C.F., BRADSHAW, C.M. & SZABADI, E. (2006): Effects of 5-HT_{2A} receptor stimulation on the discrimination of durations by rats. *Behavioural Pharmacology*, in press

Published abstracts of conference presentations

- ASGARI, K., RICKARD, J.F., ZHANG, Z., BODY, S., FONE, K.C.F., BRADSHAW, C.M. & SZABADI, E. (2004) Evidence that 5-HT_{2A} but not 5-HT₃ receptor stimulation disrupts temporal discrimination performance in rats. *Journal of Psychopharmacology* 18, A41
- BODY, S., ASGARI, K., RICKARD, J.F., ZHANG, Z., FONE, K.C.F., BRADSHAW, C.M. & SZABADI, E. (2004) Effect of quipazine on free-operant timing behaviour: evidence for involvement of 5-HT_{2A} receptors. *Journal of Psychopharmacology* 18, A42
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CHAPTER 1

INTRODUCTION

1.1. OVERVIEW OF THE PROJECT

All behaviour happens in the continuous stream of time, and all behaviours that begin and end in a point of time have temporal properties. It has been argued that understanding time is the cornerstone for understanding learning, decision making, and memory (Gibbon 1991). Two basic forms of timing are ‘endogenous rhythms’ (e.g. circadian oscillations) and interval timing (behaviour that comes under the control of temporal regularities in the environment as a result of learning) (Gibbon et al. 1997b). In cognitive terms, interval timing includes the perception, anticipation, and estimation of durations. According to Gibbon and his collaborators, appreciation of duration can be viewed as the core of the learning process (Gallistel and Gibbon 2000).

There have been many attempts to devise theoretical accounts of timing behaviour. The dominant approach for well over half a century has been based on the analogy between the behaviour of an animal or human being performing a timing task and the operation of a man-made clock. Psychologists have noticed many points of similarity between interval timing behaviour and the action of clocks, and have proposed that timing behaviour may be viewed as the product of an ‘internal clock’.

The concept of an internal clock goes back to the first half of the twentieth century (Hoagland 1933). In subsequent decades, Treisman (1963), Wing and Kristofferson (1973), and finally Gibbon (1977), proposed more sophisticated formal mathematical models for the hypothetical internal clock. For some authors, the hypothetical internal clock is simply a useful concept that allows one to build mathematical models of interval timing (e.g. Killeen et

al. 1997), whereas for others, the components of the internal clock are assumed to have biological reality that may be identified with specific neuroanatomical structures (Gibbon et al. 1997a; Matell and Meck 2004).

The present project is concerned with the psychopharmacology of interval timing behaviour. The project arose from previous work carried out on the role of 5-hydroxytryptaminergic (5-HTergic) pathways in interval timing behaviour (see Ho et al. 2002). The overall aim of the project was to extend our understanding of 5-HT's putative role in interval timing by providing answers to specific questions raised by this previous research. Most of the experiments described in this thesis entailed systemic treatment with more or less selective agonists and antagonists of particular 5-HT receptors to rats that had been trained in various types of timing task. The aim of the project was to examine the effect of manipulating the 5-HTergic system on timing performance, and not to test any particular theory of interval timing. However, some of the results obtained in the project do have implications for internal clock models, and these implications will be discussed in the thesis.

This introductory chapter starts with an overview of the structure and function of the 5-HTergic system of the brain. Many varied behavioural functions are believed to depend, at least in part, on the 5-HTergic system. No attempt will be made to provide an exhaustive review of these functions. However, a few of the functions that may have some relevance to the main topic of the thesis will be discussed.

The overview of 5-HT is followed by a review of the methodology used to study interval timing in animals, and an account of the major current theories of interval timing.

The final section of the Introduction contains a review of the literature on the behavioural pharmacology of interval timing behaviour. Emphasis is given to previous studies of the putative role of the 5-HTergic system in timing behaviour, because these studies form the immediate background to the present project.

1.2. THE 5-HYDROXYTRYPTAMINERGIC SYSTEM

1.2.1. Anatomy of the 5-hydroxytryptaminergic pathways

5-Hydroxytryptamine (5-HT, serotonin) was first isolated as a substance with vasoconstrictor properties that is present in the gastrointestinal tract by Erspamer and Asero (1953), who gave it the name “enteramine”. The “enteramine” molecule was identified as 5-hydroxytryptamine by Twarog and Page (1953), who detected it in some brain areas. These authors gave the substance the name “serotonin” due to its vasoconstrictor effects (see Deakin 1983).

Dahlstrom and Fuxe (1964) were the first pioneers in direct visualization of serotonergic (5-HTergic) neurones using histochemical fluorescence, by treating brain sections with formaldehyde. Dahlstrom and Fuxe’s work enabled the central 5-HTergic pathways to be mapped. It was discovered that the cell bodies of the central 5-HTergic neurones are located in the midline (raphe) nuclei of the brainstem. 5-HTergic neurons in the raphe nuclei are comprised of two distinct groups; the superior group is located at the boundary between midbrain and pons, and the inferior group extends from the

caudal pons to the cervical spinal cord. The dorsal raphe nucleus (DRN) and median raphe nucleus (MRN) have been classified as part of the superior raphe nuclear group; they are equivalent to groups B7 and B8 according to Dahlstrom and Fuxe's (1964) classification (see Deakin 1983; Harding et al. 2004).

The anatomical organization of the 5-HTergic pathways has been extensively studied in the cat and the rat (Leger et al. 2001; Harding et al. 2004). There are at least five separate ascending pathways to the forebrain, and three pathways projecting to the spinal cord. In the rat, the main ascending projection pathways are contained within the medial forebrain bundle, which carries unmyelinated, slender fibres from both the MRN and DRN to many areas of the brain (see Azmitia et al 2002; Harding et al. 2004); a simplified diagram of these projections is shown in Figure 1.1.

Neurones in the DRN and MRN differ somewhat in their topography; those in the DRN appear to be relatively condensed, while in the MRN they appear to be more loosely organized (Adell et al. 2002). Differences in axon morphology between fibres originating in the DRN and MRN have been reported by Murphy (1991), who found that the axons projecting from the DRN to the frontal cortex are very fine, having small varicosities, and are highly vulnerable to damage, while axons extending from the MRN to the hippocampus and lateral hypothalamus have large varicosities and are relatively resistant to the effects of selective neurotoxins (see below).

The forebrain projection regions of the DRN and MRN show considerable overlap. Many areas, for example the amygdala and parts of the neocortex, receive equivalent inputs from both nuclei (Imai et al. 1986;

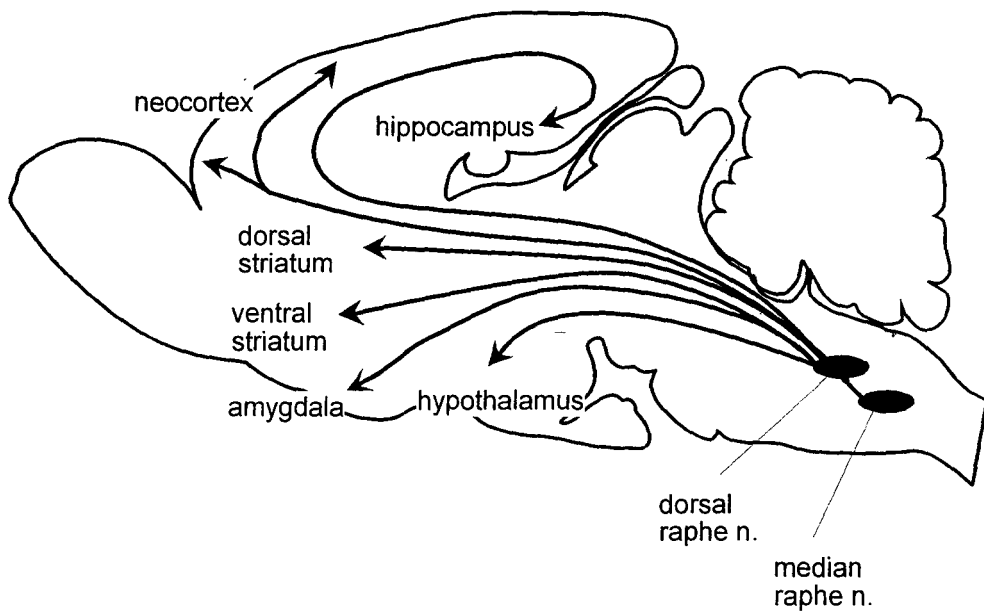


Figure 1.1. The ascending 5-HTergic pathways of the rat brain.

Neuman et al. 1992; Van Bockstaele et al. 1993). However, the dorsal striatum receives nearly all of its 5-HTergic innervation from the DRN, whereas the dorsal hippocampus is innervated almost exclusively by the MRN (Imai et al. 1986). Consistent with these neuroanatomical findings, studies of 5-HT release have also indicated partial segregation of the 5-HTergic projection from the DRN and MRN. McQuade and Sharp (1997) measured extracellular 5-HT in brain regions, using microdialysis, following stimulation of the two nuclei. Stimulation of the DRN provoked 5-HT release in the frontal cortex, dorsal striatum and globus pallidus, while stimulation of the MRN provoked 5-HT release in the dorsal hippocampus. Stimulation of either nucleus resulted in 5-HT release in the medial septum and ventral hippocampus.

The raphe nuclei also project to other brainstem nuclei. There are 5-HTergic projections to both the noradrenergic and the dopaminergic nuclei. Both the DRN and MRN contribute to these projections (Harding et al. 2004). Interestingly, both the DRN and MRN receive noradrenergic and dopaminergic input (Adell et al. 2002; Harding et al. 2004). The noradrenergic afferents to the raphe nuclei arise mainly from the locus coeruleus, although other neighbouring noradrenergic nuclei also contribute (Peyron et al. 1996). Neurones in both the DRN and MRN express α_1 -adrenoceptors and α_2 -adrenoceptors. There is evidence that stimulation of α_1 -adrenoceptors can facilitate 5-HTergic transmission (Baraban and Aghajanian 1980), whereas α_2 -adrenoceptor stimulation suppresses the activity of 5-HTergic neurones (Adell and Artigas 1999). The dopaminergic input to the raphe nuclei arises from both the substantia nigra and the ventral tegmental area (Beckstead et al. 1979). The dopaminergic influence on neuronal activity in the raphe nuclei is mainly

facilitatory, and is mediated primarily by D₂ dopamine receptors (Adell et al. 2002).

1.2.2. **Synthesis and metabolism of 5-hydroxytryptamine**

The basic biochemical mechanisms involved in the synthesis and degradation of 5-HT have been known for many years (Costa and Meek 1974). Only a brief summary is provided in this section.

The synthesis of 5-HT begins from its precursor, the amino-acid tryptophan. Tryptophan is obtained from ingested food. However, normal diet generally contains lower amounts of tryptophan than of other large neutral amino-acids (e.g. tyrosine). The capillary endothelia that comprise the blood-brain barrier contain a limited-capacity transport system for neutral amino-acids; this is a 'passive' process (i.e. one that is not energy-dependent: Wurtman et al. 1981). Neutral amino-acids compete for passage through the blood-brain barrier via this transport process. Thus, a high dietary loading of other neutral amino-acids results in a reduction of brain tryptophan levels. This is the basis of the 'acute tryptophan depletion' procedure that has been widely used in studies of 5-HTergic function in man (Delgado et al. 1990). There is a diurnal variation in tryptophan levels in the plasma (lower at midday, and higher in the evening), which is mainly due to the consumption of meals. Insulin secretion following the ingestion of a carbohydrate-rich meal results in lowering of the plasma levels of most neutral amino-acids; however tryptophan levels are not significantly affected, due to the binding of tryptophan to albumin in the plasma. The net result of this is that the plasma tryptophan *ratio*

(i.e. the ratio of tryptophan to other amino-acids) is increased, and this results in increased penetration of tryptophan into the brain (Wurtman et al. 1981).

Tryptophan is taken into 5-HTergic neurones by an energy-dependent process. The specific intracellular enzyme tryptophan hydroxylase converts tryptophan to 5-hydroxytryptophan. This is the rate-limiting step in 5-HT synthesis; inhibition of the enzyme by para-chlorophenylalanine (PCPA) results in long-lasting 5-HT depletion in the brain (Costa and Meek 1974).

5-Hydroxytryptophan is converted to 5-HT by the non-specific enzyme L-aromatic amino-acid decarboxylase. 5-HT, like other neurotransmitters, is retained in vesicles within the nerve terminals, which protect it from enzymatic degradation (see below).

Like many other transmitters, the release of 5-HT into the synaptic cleft is accomplished by a calcium-dependent process following depolarization of the membrane of the presynaptic terminal. 5-HT's action at post-synaptic receptors is considered in the following section.

Following release, 5-HT is taken back into the 5-HTergic neurone by an energy-dependent transport process. The serotonin transporter (SERT) molecule which is responsible for this process has been found in all areas of the brain that receive a 5-HTergic projection (Gainetdinov and Caron 2003). The uptake process can be inhibited by tricyclic antidepressants (which also inhibit noradrenaline uptake), and by 'selective serotonin reuptake inhibitors' (SSRIs) (see Szabadi and Bradshaw 2004).

After reuptake, 5-HT is degraded by the mitochondrial enzyme monoamine oxidase (MAO), which deaminates 5-HT and other monoamines. In the case of 5-HT, the resulting metabolite is 5-hydroxyindoleacetic acid (5-

HIAA). There are two forms of MAO; MAO-A and MAO-B. Although 5-HT is a better substrate for MAO-A than MAO-B, 5-HT cell bodies in the brain contain greater amounts of MAO-B than MAO-A (Azmitia et al. 2002). Monoamine oxidase inhibitors (MAOI) can block the destruction of 5-HT, thereby increasing the synaptic availability of 5-HT (see Szabadi and Bradshaw 2004).

1.2.3. **5-hydroxytryptamine receptors**

1.2.3.1. *Historical background*

5-HT mediates a wide range of physiological functions in both the central nervous system (CNS) and in the periphery. These functions are mediated by multiple types of receptor. Early attempts to define and classify these receptors, starting in the 1950s, necessarily relied on the functional pharmacological methods that were available at that time. The introduction of radioligand binding techniques, second messenger assays, and, more recently, cloning techniques, have resulted in the discovery of an increasing number of receptor subtypes.

Peripheral 5-HT receptors were classified into two major subtypes by Gaddum and Picarelli (1957). These authors demonstrated that 5-HT-induced contractions of the guinea-pig ileum could be blocked partly by morphine and partly by dibenzylamine. This led them to conclude that the contractile response might be mediated by two different receptor populations, which they named M-

and D-receptors, respectively (see Hoyer et al. 2002; Lanfumey and Hamon 2004).

In 1979, Perutka and Snyder, using radioligand binding techniques, identified two distinct 5-HT binding sites in brain tissue, which they designated 5HT₁ and 5-HT₂ receptors. 5-HT₁ receptors were found to have high affinity for 5-HT, and 5HT₂ receptors to have lower affinity for 5-HT, but higher affinity for 5-HT antagonists. The peripheral M-receptors were thought to be distinct from 5-HT₁ receptors, but the D-receptors were thought to be pharmacologically similar to 5-HT₂ receptors (Lanfumey & Hamon 2004; Hoyer et al 2002). It soon became apparent that 5-HT₁ receptors are heterogeneous and can be divided into several subgroups (see below).

Interest in the M-receptor increased with the discovery of selective antagonists for this site (Fozard and Gittos 1983). The receptor was formally re-named the 5-HT₃ receptor in an internationally agreed taxonomy of 5-HT receptors (Bradley et al. 1986).

To date, at least seven different types of 5-HT receptor have been identified, several of them have been described with multiple subtypes (Glennon et al. 2002). Except for the 5HT₃ receptor, which belongs to the ‘superfamily’ of ligand-gated ion channels, all other 5-HT receptor types belong to the G protein-coupled receptor ‘superfamily’. The widespread use of binding and cloning techniques has led to the identification of a large number of binding sites, and it is not clear how many of them may eventually be attributed to the 5-HT receptor family. Therefore, an international committee has proposed a new system of classification, in which the term 5-HT receptor can be applied only when three classes of operational criteria - structural,

transductional and functional information - are available for that receptor (Hoyer et al. 1994). Some of the newly discovered recognition sites for 5-HT, which are not coupled to any known physiological mechanism, have been named with lower case designations (e.g. 5-ht_{1F} and 5-ht_{5A}).

The aim of this section is to provide a synopsis of the principal subtypes of 5-HT receptors, including their neuroanatomical distribution and pharmacological features. The behavioural roles of the receptors are mainly discussed in the following section. As well as the original papers cited below, this synopsis draws heavily on recent major reviews of 5-HT receptors, including Barnes and Sharp (1999), Glennon et al. (2002), and Hannon and Hoyer (2002), and Hoyer et al. (2002). The chemical names of compounds identified by pharmaceutical company codes are only provided in the case of drugs that have relevance to later chapters of the thesis; a comprehensive list of chemical names is given in the Appendix (see also Hoyer et al. 2002).

1.2.3.2. *The 5-HT₁ receptor family*

This group of receptors was first subdivided into six subtypes, designated 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F}. Subsequently, the 5-HT_{1C} receptor was re-assigned to the 5-HT₂ receptor family and re-named 5-HT_{2C}, because it was found to have close homology with other 5-HT₂ receptors both in terms of DNA coding and second messenger coupling (Lanfumey and Hamon 2000, 2004). Little is known about 5-HT_{1E} and 5-HT_{1F} receptors, and therefore many authors still prefer the lower-case designation 5-ht_{1E} and 5-ht_{1F} (Pauwels 2003).

5-HT₁ receptors are the largest class of 5-HT receptors, and they have high affinity for 5-HT. Like all other 5-HT receptors except the 5-HT₃ receptor, they belong to the G-protein-coupled superfamily. 5-HT₁ receptors inhibit adenylyl cyclase when they are stimulated.

1.2.3.2.1. 5-HT_{1A} receptors

These receptors can be found in many parts of the CNS. They occur in large numbers in the median and dorsal raphe nuclei. The great majority of these receptors are located on the somata and dendrites of 5-HTergic neurones, although a significant minority are expressed by non-5-HTergic cells (Kirby et al. 2003). The 5-HT_{1A} receptors on 5-HTergic neurones are believed to function as inhibitory autoreceptors. Stimulation of these receptors inhibits cell firing in the raphe nuclei.

5-HT_{1A} receptors are also abundant in many other parts of the CNS, especially the limbic system, where they function as postsynaptic receptors. They are expressed most densely in the hippocampus, and are also found in the septum and amygdala. Somewhat lower concentrations are found in the prefrontal and entorhinal cortex, and some nuclei of the thalamus and hypothalamus. They are barely detectable in the basal ganglia and cerebellum (see Barnes and Sharp 1999).

5-HT_{1A} receptors are negatively coupled to the adenylyl cyclase second messenger system (Glennon et al. 2002; Hoyer et al. 2002). The most widely used full agonist of 5-HT_{1A} receptors is 8-hydroxy-(di-n-propylamino)tetralin (8-OH-DPAT). The anxiolytic buspirone, and its close relatives ipsapirone and

gepirone, are rather selective partial agonists at these receptors. *N*-[2-(4-[2-methoxyphenyl]-1-piperazinyl)ethyl]-*N*-2-pyridinylcyclohexane-carboxamide (WAY-100635) is a highly selective antagonist with high affinity for 5-HT_{1A} receptors (Pauwels 2003).

1.2.3.2.2. 5-HT_{1B} receptors

5-HT_{1B} receptors can be found in many areas of the CNS. In the globus pallidus, substantia nigra and parts of the frontal cortex, they act as terminal autoreceptors on 5-HTergic neurones, controlling the release of 5-HT. 5-HT_{1B} receptors are also thought to act as terminal heteroreceptors on non-5-HTergic neurones, controlling the release of acetylcholine, glutamate, dopamine, noradrenaline, and γ -aminobutyric acid (GABA) (Barnes and Sharp 1999; Hoyer et al. 2002; Boothman et al. 2003; Pauwels 2003). These receptors have also been found in arteries of the cerebral circulation, where they mediate the vasoconstrictor effect of 5-HT (Hoyer et al 2002; Lanfumey and Hamon 2004).

5-HT_{1B} receptors are negatively coupled to adenylate cyclase. The first reported full agonist at 5-HT_{1B} receptor was RU-24969. The anti-migraine drug sumatriptan, and the closely related compounds rizatriptan and zolmitriptan, are partial agonists at these receptors. These compounds have more or less equal affinity for 5-HT_{1B} and 5-HT_{1D} receptors (see below). Other 5-HT_{1B} receptor agonists include the 'serenics' (e.g. eltoprazine), which were developed as potential anti-aggressive agents, but were found to be ineffective in controlling aggression in clinical trials (de Koning et al. 1994). There are no entirely satisfactory 5-HT_{1B} receptor antagonists available at present. The β -

adrenoceptor antagonists pindolol and cyanopindolol are effective antagonists at these receptors, as is the non-selective 5-HT₁ receptor antagonist methiothepin (see Barnes and Sharp 1999; Hoyer et al. 2002). SB-216641 and SB-224289 are among the few compounds which discriminate between 5-HT_{1B} and 5-HT_{1D} receptors (Price et al. 1997); they are inverse agonists at 5-HT_{1B} receptors (Roberts et al. 2000).

1.2.3.2.3. 5-HT_{1D} receptors

The receptor originally designated as 5-HT_{1D} is almost completely absent in the rat brain, but has a distribution in the human and guinea-pig brain that closely resembles the distribution of 5-HT_{1B} receptors in the rodent brain. The situation is complicated by the fact that there are two distinct forms of the 5-HT_{1D} receptor, originally designated 5-HT_{1Dα} and 5-HT_{1Dβ}, which display about 77% sequence homology (see Glennon et al. 2002). The 5-HT_{1Dα} is, in fact, present both in rodents and in humans, whereas the 5-HT_{1Dβ} receptor (which is one and the same as the original 5-HT_{1D} receptor) is absent in rodents. The taxonomy of these receptors has now been simplified by identifying the 5-HT_{1B} and 5-HT_{1Dβ} receptors as species variants of a single receptor subtype. According to the current nomenclature, these species variants are collectively known as the 5-HT_{1B} receptor, and the name '5-HT_{1D}' is now reserved for the 5-HT_{1Dα} receptor (Barnes and Sharp 1999; Pauwels 2003).

5-HT_{1D} receptors have been identified predominantly in the caudate-putamen, and to lower degrees in other parts of CNS, especially the olfactory tubercle, entorhinal cortex, dorsal raphe nucleus, and cerebellum (see Pauwels

2003; Lanfumey and Hamon 2004). These receptors are G protein-linked, and are coupled to inhibition of adenylyl cyclase. They are believed to contribute (together with 5-HT_{1B} receptors) to the inhibitory regulation of 5-HT release, and also to mediate the inhibitory effect of 5-HT on glutamate and somatostatin release (Maura et al 1998; see Lanfumey and Hamon 2004).

As indicated above, sumatriptan is an agonist at 5-HT_{1D} receptors, but also binds to 5-HT_{1B}, and to some extent to 5-HT_{1A} receptors. PNU 109291 shows greater selectivity for the 5-HT_{1D} receptor (Pauwels 2003). 5-HT_{1D} receptor antagonists include GR127935 and GR55562, although these do not show good selectivity for 5-HT_{1D} over 5-HT_{1B} receptors (Glennon et al. 2002).

1.2.3.2.4. 5-HT_{1E} receptors

Since it is not confirmed that these receptors have a true physiological role, they retain their lower case appellation. Like other members of 5-HT₁ family subtypes, this receptor is coupled negatively to adenylyl cyclase. In terms of their structure, there is considerable sequence homology between 5-HT_{1E} and 5-HT_{1D} receptors, leading to the suggestion that the two receptor subtypes belong to the same group (Glennon et al. 2002). However they differ in their affinity for ligands; 5-carboxytryptamine has very low affinity for the 5-HT_{1E} binding site, in contrast to its high affinity for the 5-HT_{1D} site (Lanfumey and Hamon 2004). There is evidence for slight differences between the human and rodent 5-HT_{1E} receptors, which have been labelled 5-HT_{1Eα} and 5-HT_{1Eβ}, respectively (Glennon et al. 2002).

The 5-HT_{1E} receptor has been found in the frontal cortex, but a comprehensive mapping of its distribution in the brain has not yet been undertaken. Selective agonists and antagonists for this receptor are not yet available (see Pauwels 2003; Lanfumey and Hamon 2004).

1.2.3.2.5. 5-HT_{1F} receptors

This receptor is thought to be closely related to 5-HT_{1E} receptor, because the two subtypes exhibit considerable sequence homology (>70%; see Hoyer et al. 2002). Antimigraine drugs, for example sumatriptan, label 5-HT_{1F} receptors with high affinity, and this property distinguishes the 5-HT_{1F} receptor from the 5-HT_{1E} receptor (Barnes and Sharp 1999). It has been suggested that the 5-HT_{1F} receptor, rather than the 5-HT_{1D} receptor, may be the mediator of the therapeutic effects of these drugs (Hamon and Bourgoin 2000). 5-HT_{1F} receptor mRNA has been found in the dorsal raphe nucleus, hippocampus, cerebral cortex, striatum, thalamus and hypothalamus (see Lanfumey and Hamon 2004).

5-HT_{1F} receptors are negatively coupled to adenylyl cyclase. Two putative selective agonists have been described: LY-344864 and LY-334370 (see Pauwels 2003).

1.2.3.3. *The 5-HT₂ receptor family*

This class of 5-HT receptors consists of three subtypes: 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}. The last of these was formerly termed 5-HT_{1C} (see above). Like the 5-HT₁ family, this class belongs to the metabotropic receptor 'superfamily'. All

three subtypes of 5-HT₂ receptor are coupled to the phosphoinositide system, receptor stimulation resulting in an increase in inositol phosphate hydrolysis (see Hoyer et al. 2002). The three receptors in this class show a high sequence similarity; the homology within the transmembrane domains has been reported as more than 70% (Hoyer and Martin 1997; Glennon et al. 2002; Leysen 2004).

1.2.3.3.1. 5-HT_{2A} receptors

The 5-HT_{2A} receptor has been identified with the classical D receptor of Gaddum and Picarelli (1957), which was later defined by Peroutka and Snyder (1979) as the 5-HT₂ receptor. These receptors have been found in almost all parts of the brain. They are especially abundant in the telencephalon (including the olfactory system, cerebral cortex, hippocampus, amygdala and corpus striatum), but are also present in considerable numbers in the diencephalon (dorsal thalamus and hypothalamus), mesencephalon (substantia nigra and ventral tegmental area), metencephalon (latero-dorsal tegmental nucleus), and on spinal cord motoneurons; they have also been found on sympathetic and sensory ganglion neurones (Barnes and Sharp 1999; Hoyer et al. 2002). The distribution of 5-HT_{2A} receptors indicates that they are postsynaptically located in structures innervated by the 5-HTergic projection (Blue et al. 1988). They have been localized on GABAergic interneurons and on the apical dendrites of glutamatergic projection neurones of the cerebral cortex (see Barnes and Sharp 1999). It has been proposed that 5-HT_{2A} receptors have a 'balancing' role in the regulation of inhibitory and excitatory signalling in the cortex; for example their activation may promote GABA-mediated inhibition of

glutamatergic pyramidal neurones and may simultaneously produce a direct excitatory effect on the pyramidal cells (see Leysen 2004).

There are no known selective agonists for the 5-HT_{2A} receptor. DOI (2,5-dimethoxy-4-iodoamphetamine) has been widely used as a 5-HT_{2A} receptor agonist; however this agent also has a high affinity for 5-HT_{2C} receptors. Amongst the antagonists, ketanserin has an 80-fold selectivity for 5-HT_{2A} over 5-HT_{2C} receptors (with little affinity for 5-HT_{2B} receptors) (Baxter et al. 1995). MDL 100907 (2,3-dimethoxyphenyl-1-[2-(4-piperidine)-methanol]) is even more selective, having an affinity for the 5-HT_{2A} receptor that is almost two-and-a-half orders of magnitude greater than its affinity for the 5-HT_{2C} receptor (Kehne et al. 1996; see Barnes and Sharp 1999; Glennon et al. 2002; Hoyer et al. 2002; Leysen 2004).

1.2.3.3.2. 5-HT_{2B} receptors

5-HT_{2B} receptors were first described in the rat stomach fundus, and then were identified in human gastrointestinal tract, particularly in the colon, and in the myenteric plexus where they mediate contractile functions. They have also been found on endothelial cells of the cerebral arteries where they cause vascular relaxation by releasing nitric oxide. Their presence in the brain appears to be restricted to the cerebellum, lateral septum, hypothalamus and medial amygdala (Hamon and Hoyer 2002; Leysen 2004). 5-HT_{2B} receptors in the brain are coupled to phosphoinositol hydrolysis (Barnes and Sharp 1999). Interestingly there is a high homology (>90%) between rat and human 5-HT_{2B} receptors (Hoyer and Martin 1997; Leysen 2004).

Selective agonists (e.g. BW-723C86) and antagonists (SB-200464) are available for the 5-HT_{2B} receptor (see Pauwels 2003; Leysen 2004).

1.2.3.3.3. 5-HT_{2C} receptors

This subtype was the first of 5-HT₂ receptor family to be cloned, and was once referred to as the 5-HT_{1C} receptor. This receptor was identified in the choroid plexus of various species, suggesting that it may have a role in the regulation of cerebrospinal fluid (Kaufman et al. 1995). 5-HT_{2C} receptors have also been found in the pyriform and cingulate cortices, parts of the limbic system (nucleus accumbens, amygdala and hippocampus) and basal ganglia (caudate nucleus, substantia nigra) (Palacios et al. 1991; Barnes and Sharp 1999). Their presence on GABAergic neurones in the basal ganglia has led to the suggestion that they may exert an indirect influence on dopaminergic transmission (Eberle-Wang et al. 1996, 1997). In keeping with this suggestion, 5-HT_{2C} receptor antagonists have been reported to increase extracellular dopamine levels in the basal ganglia (Pozzi et al. 2002).

5-HT_{2C} receptors are coupled to phosphoinositol hydrolysis. No highly selective agonists have been described. As indicated above, DOI has similar affinity for 5-HT_{2A} and 5-HT_{2C} receptors; mCPP (2-(2-methyl-4-chlorophenoxy)propanoic acid) has affinity for 5-HT_{2B} as well as 5-HT_{2C} receptors. Among its antagonists, SB-242084 and RS-102221 are the most selective (Pauwels 2003; Leysen 2004).

1.2.3.4. 5-HT₃ receptors

The 5-HT₃ receptor is the same as Gaddum and Picarelli's (1957) "M" receptor. It is unique among the families of 5-HT receptors in belonging to the ligand-gated ion channel (ionotropic) receptor superfamily. 5-HT₃ receptor stimulation produces a depolarising response, due to non-selective cation channel opening. 5-HT₃ receptors are found both in the CNS (area postrema, enthorinal cortex, frontal cortex, and hippocampus) and in the periphery (gastrointestinal tract, cardiovascular system). Three subunits of the 5-HT₃ receptor have been identified, designated 5-HT_{3A}, 5-HT_{3B} and 5-HT_{3C} (see Costall and Naylor 2004).

5-HT₃ receptors have been localized on GABAergic neurones in the basal ganglia, and 5-HT₃ receptor stimulation has been reported to promote GABA release (Morales and Bloom 1997). 5-HT₃ receptor agonists also promote dopamine release in the nucleus accumbens; the location of the 5-HT₃ receptors responsible for this effect is not known (DeDeurwaerdere et al. 1998).

Selective 5-HT₃ receptor agonists include 2-methyl-5-HT, phenylbiguanide, and m-chlorophenylbiguanide (mCPBG), mCPBG having the highest affinity (within the nanomolar range) (Kilpatrick et al. 1990). Quipazine, which has nanomolar affinity for 5-HT₃ receptors, also has relatively high affinity (micromolar range) for 5-HT_{2A} receptors (Hoyer 1988; Glennon et al. 1989; Sharif et al. 1991). MDL-72222 (tropanyl 3,5-dichlorobenzoate) was the first potent and selective 5-HT₃ receptor antagonist to be described (Fozard and Gittos 1983). Other selective antagonists for 5-HT₃ receptors include ondansetron, tropisetron and granisetron; these compounds are efficacious in suppressing nausea and vomiting induced by cytotoxic

agents, this effect being mediated by blockade of 5-HT₃ in the area postrema and possibly also on vagal afferents in the gut (Andrews 1994; Costall and Naylor 2004).

1.2.3.5. *The 5-HT₄ receptor family*

The 5-HT₄ receptor was first identified in cultured neurones from the mouse colliculi (Bockaert et al. 1990). The application of cloning technology revealed that there are numerous variants (eight, according to a recent review: Bockaert et al. 2004), labelled 5-HT_{4a}, 5-HT_{4b}, etc. These variants show subtle differences in binding affinities for agonists and antagonists, and subtly different regional distribution in the brain. However, the degree of pharmacological specificity shown by currently available ligands has proved insufficient to identify clearly distinct functional profiles (see Bockaert et al. 2004). Therefore for the purposes of this brief synopsis, they will be treated as though they constituted a unitary receptor population.

5-HT₄ receptors are metabotropic receptors that are positively coupled to adenylyl cyclase. They occur in the periphery and in the CNS. In the periphery, they are located mainly in the gastrointestinal tract, where they increase gut motility, apparently via acetylcholine release (Buchheit et al. 1995). They are widely distributed in the CNS, high concentrations being found in limbic structures (nucleus accumbens, amygdala), olfactory tubercle, hippocampus, basal ganglia (striatum, globus pallidus), substantia nigra and hypothalamus (Bockaert et al. 2004). 5-HT₄ receptors in the striatum have been localized on the somata and dendrites of GABAergic neurones, including those

that project to the substantia nigra and globus pallidus (Compan et al. 1996). 5-HT₄ receptors in the hippocampus and prefrontal cortex have been localized on both cholinergic and glutamatergic neurones (Matsumoto et al. 2001). 5-HT₄ receptor agonists promote dopamine release in the dorsal striatum (Lucas et al. 2001), and acetylcholine release in the frontal cortex (Consolo et al. 1994). An interesting feature of 5-HT₄ receptors is that they show varying degrees of 'constitutive' activity, that is, they can be functionally active in the absence of any agonist stimulation. An implication of this property is that antagonists may act as inverse agonists, depending on the level of constitutive activity (see Bockaert et al. 2004).

No full agonists have yet been described. High-affinity partial agonists include RS-67333 and RS-67506; GR113808 and GR125487 are selective antagonists (Bockaert 2004).

1.2.3.6. *The 5-HT₅ receptor family*

The 5-HT₅ receptor has been classified as an 'orphan' receptor (Hoyer et al. 2002), due to lack of evidence for any physiological role for this binding site. Two subtypes have been cloned, 5-HT_{5A} and 5-HT_{5B}, which have been found in the mouse and rat respectively, and share 70% overall sequence identity (see Pauwels 2003). The 5-HT_{5B} receptor is not expressed in humans (Nelson 2004; Hannon and Hoyer 2002). Some authors reported that, in the rat, 5-HT_{5A} receptor may be negatively coupled to adenylyl cyclase; however, it has also been reported that this receptor may couple positively to cyclic AMP production (see Hoyer et al. 2002). 5-HT₅ receptors have been found in the

olfactory bulb, caudate-putamen, neocortex, hippocampus, hypothalamus, and cerebral ventricles (Nelson 2004; Hannon and Hoyer 2002). At present there are no selective agonists or antagonists for these receptors (Pauwels 2003).

1.2.3.7. *The 5-HT₆ receptor family*

The 5-HT₆ receptor in the rat is positively coupled to adenylyl cyclase, and shares <40% sequence homology with all other 5-HT receptors that couple to G-proteins (Wooley et al. 2004). It is expressed almost exclusively in the brain. The richest area for 5-HT₆ receptors is the nucleus accumbens, and the poorest area is cerebellum; 5-HT₆ receptors are also found in the hippocampus, olfactory tubercle, striatum and cortex (Wooley et al. 2004; Hoyer et al. 2002). The 5-HT₆ receptor has a high affinity for both typical and atypical antipsychotics; Wooley et al. (2004) suggested that the preponderant location of this receptor in the ventral striatum (nucleus accumbens) is consistent with a potential role in the mode of action of antipsychotics. The receptors are apparently located on postsynaptic structures, because destruction of the 5-HTergic pathways does not reduce the receptor population in the forebrain (Gerald et al. 1996). SB-271046 and Ro 04-6790 are reported to be selective and potent antagonists at 5-HT₆ sites; no highly selective agonists are available as yet (Hoyer et al. 2002; Wooley et al. 2004).

1.2.3.8. *The 5-HT₇ receptor family*

5-HT₇ receptors are coupled positively to adenylyl cyclase via G-proteins. They have been found in CNS, and also in the peripheral tissue. To date a number of subtypes have been reported for 5-HT₇ receptors: 5-HT_{7(a)}, 5-HT_{7(b)}, and 5-HT_{7(c)} in the rat, and 5-HT_{7(a)}, 5-HT_{7(b)}, and 5-HT_{7(d)} in the human brain (Thomas and Hagan 2004). These receptors are expressed in thalamus, hypothalamus, cerebral cortex and amygdala. The receptors are believed to be postsynaptic. The hypothalamic receptor population is particularly dense in the suprachiasmatic nucleus, which has been implicated in the control of the circadian rhythm and hormone release (Hedlund and Sutcliffe 2004).

No selective 5-HT₇ agonists exist at present. The 5-HT_{1A} receptor agonist 8-OH-DPAT has a high affinity for the 5-HT₇ receptor; however, the 5-HT_{1A} receptor antagonist WAY-100635 has very little affinity for the 5-HT₇ receptor. Selective antagonists for the 5-HT₇ receptor include SB 258719, and DR 4004 (Hedlund and Sutcliffe 2004; Thomas and Hagan 2004).

1.2.4. Behavioural role of the 5-hydroxytryptaminergic system

The behavioural role of the 5-HTergic system includes a very broad range of behaviours, from sleep, locomotor activity, feeding, sexual behaviour, aggression and anxiety, to complex learned behaviours such as inter-temporal choice, memory and timing. Dysfunction of the 5-HTergic system has been implicated in a range of psychiatric disorders, including generalized anxiety disorder, obsessive-compulsive disorder, depression, schizophrenia and pathological impulsiveness. In this section, some behavioural roles of 5-HT that have potential relevance to the experimental work described in this thesis

will be outlined. Previous studies of the role of 5-HT in timing behaviour will be reviewed separately in section 1.5. (*Behavioural pharmacology of timing*).

1.2.4.1. *5-HT and sleep/arousal*

The possible role of the 5-HTergic pathways in sleep and arousal was first suggested by Bradley's (1958) observation that intraventricularly administered 5-HT to cats induced a brief increase in arousal followed by prolonged somnolence. Subsequently it was shown that central 5-HT depletion by *p*-chlorophenylalanine suppressed sleep in cats (Koella et al. 1968). Jouvett (1972) showed that loss of 5-HTergic function had an especially marked effect on 'paradoxical' or rapid-eye-movement (REM) sleep. Jouvett (1972) proposed that 5-HT might serve to induce sleep. However, this theory was contradicted by the finding that 5-HTergic neurones of the dorsal raphe nucleus were most active during wakefulness, less active during slow-wave sleep, and quiescent during REM sleep (e.g. Lydic et al. 1987). Consistent with this finding, microdialysis studies have shown that extracellular 5-HT in the raphe nuclei is at its highest level during wakefulness, lower during REM sleep and minimal during slow wave sleep (Portas et al. 1998, 2000). Suppression of 5-HTergic activity by intra-raphe injection of the 5-HT_{1A} receptor agonist 8-OH-DPAT (thus stimulating 5-HT release-inhibiting autoreceptors: see above) induces REM sleep (Portas et al. 1996; Bjorvatn et al. 1997).

The targets for the 5-HTergic neurones involved in the suppression of REM sleep have been proposed to be the pedunclopontine and laterodorsal tegmental nuclei, both of which have been implicated in the genesis of REM sleep (see Portas et al. 2000). Studies of the effects of intracerebrally injected

agonists and antagonists indicate that the postsynaptic 5-HT receptors mediating this effect include 5-HT_{1A} (Monti et al. 1994; Monti and Monti 2000) and 5-HT_{2A} (Amici et al. 2004) receptors. In addition, studies of the sleep patterns of 5-HT_{1B} receptor knockout mice, and the ability of 5-HT_{1B} receptor antagonists to induce REM sleep, indicate that this receptor subtype may also be involved in the regulation of REM sleep (Boutrel et al. 1999).

The 5-HTergic projection to the suprachiasmatic nucleus has also been implicated in the circadian sleep-wakefulness cycle, in which that nucleus is prominently involved. 5-HT's action in the suprachiasmatic nucleus appears to be mediated by 5-HT₇ receptors (Hagan et al. 2000).

The recent discovery of the important role played by histaminergic and orexinergic neurones in sleep and arousal mechanisms has tended to relegate the 5-HTergic system to a subsidiary role in current models of sleep mechanisms (see Pace-Schott and Hobson 2002; Mignot et al. 2002). For example, according to one model, 5-HTergic neurones of the raphe nuclei contribute to the inhibition of γ -aminobutyric acid (GABA)- and galanin-producing neurones of the ventrolateral preoptic nucleus, which in turn inhibit the raphe nuclei, locus coeruleus and cholinergic neurones of the pedunculopontine tegmental nucleus and laterodorsal tegmental nucleus. All these regions receive excitatory influences from orexinergic neurones of the lateral hypothalamus (see Pace-Schott and Hobson 2002). The complexities of this system are beyond the scope of this review; however it is clear that there are many questions about the contribution of 5-HTergic mechanisms to sleep and arousal that remain unresolved (see Jouvet 1999).

1.2.4.2. *5-HT and locomotor behaviour*

Central 5-HT depletion results in enhancement of spontaneous locomotor behaviour and locomotor behaviour conditioned to food presentation (e.g. Winstanley et al. 2004). In two-lever operant tasks, 5-HT depleted rats consistently switch between levers at a higher rate than normal rats (Al-Zahrani et al. 1996; see Al-Ruwaitea et al. 1997). Although this effect of 5-HT depletion may reflect locomotor activation, this is not necessarily the case, because other interventions that facilitate spontaneous locomotion (e.g. systemic treatment with *d*-amphetamine) do not promote switching (Chiang et al. 2000a).

Systemic treatment with 5-HT receptor agonists and antagonists generally has rather weak effects on spontaneous locomotor behaviour. The 5-HT_{1A} receptor agonist 8-OH-DPAT has been found to suppress locomotion (De la Garza and Cunningham 2000). 5-HT_{2A} receptor agonists and antagonists have been found to have no significant effect on spontaneous locomotion (Koskinen et al. 2000), whereas 5-HT_{2C} receptor stimulation has been found to induce hypolocomotion (Kennett and Curzon 1988). The role of 5-HT₃ receptors is unclear. For example, Mazzola-Pomietto et al. (1995) reported that the 5-HT₃ receptor agonist m-CPBG had no significant effect on spontaneous locomotion, whereas the antagonist MDL-72222 dose-dependently reduced locomotion.

In contrast to their relatively weak effects on spontaneous locomotion,

agonists and antagonists of several types of 5-HT receptor have profound effects on the hyperlocomotion induced by psychostimulant drugs. Thus, 5-HT_{2A} receptor antagonists attenuated cocaine-induced hyperlocomotion (Filip et al, 2001; McMahon and Cunningham 2001; Fletcher et al. 2002). In contrast, 5-HT_{2C} receptor antagonists potentiated cocaine-induced hyperlocomotion (Fletcher et al. 2002; Filip et al. 2004). 5-HT_{1A} receptors may also mediate attenuation of cocaine-induced locomotor stimulation. Thus, 8-OH-DPAT was found to reduce the effect of cocaine on locomotion, and the selective 5-HT_{1A} receptor antagonist blocked the effect of 8-OH-DPAT without altering the 'baseline' effect of cocaine (Przegalinski and Filip 1997).

The anatomical location of the 5-HT receptor populations mediating these effects is not clear. There is evidence that post-synaptic 5-HT_{2C} receptors exist in the nucleus accumbens and prefrontal cortex, two target regions for the mesolimbic dopaminergic projection. 5-HT_{2C} receptors in these two regions may exert opposing effects. Thus Filip and Cunningham (2002, 2003) found that injection of 5-HT_{2C} receptor agonists into the accumbens enhanced cocaine-induced locomotion, whereas injection of the same agonists into the prefrontal cortex had the opposite effect.

1.2.4.3. *5-HT and feeding*

It has been known for many years that manipulation of the 5-HTergic system has marked effects on ingestive behaviour. Destruction of the ascending 5-HTergic projection results in an increase of food intake (Hoebel et al. 1978), whereas the 5-HT-releasing agent fenfluramine (Grignaschi et al. 1992) and 5-

HT reuptake inhibitors (Lucki et al. 1988; Fletcher et al. 1993) suppress feeding behaviour. Such observations suggest that 5-HT plays a predominantly inhibitory role in the control of feeding.

Several types of 5-HT receptor have been implicated in 5-HT's role in feeding, including 5-HT_{1A}, 5-HT_{1B} and 5-HT_{2C} receptors (see De Vry and Schreiber 2000).

The 5-HT_{1A} receptor agonist 8-OH-DPAT promotes feeding behaviour (Dourish et al. 1985); this effect can be blocked by the selective 5-HT_{1A} receptor antagonist WAY-100635 (Hartley and Fletcher 1994). The effect is seen following direct injection of the agonist into the raphe nuclei, and is absent after destruction of the 5-HTergic pathways with 5,7-dihydroxytryptamine, indicating that the effect is mediated by stimulation of the somatodendritic release-inhibiting autoreceptor population of 5-HT_{1A} receptors (Bendotti and Samanin 1986). 8-OH-DPAT's facilitatory effect on feeding is seen in freely feeding rats, but not in food-deprived rats, suggesting that 8-OH-DPAT may disinhibit feeding following satiety (Dourish et al. 1985). Consistent with this interpretation, Voigt et al. (2000) showed that 5-HT release in the lateral hypothalamus (an area that plays a prominent role in ingestive behaviour) was enhanced by 8-OH-DPAT in freely feeding rats, but not in food-deprived rats.

There is good evidence for an important role of 5-HT_{1B} receptors in feeding. The non-selective 5-HT_{1B/2C} receptor agonist 1-(*m*-chlorophenyl)-piperazine (*m*CPP) and the selective 5-HT_{1B} receptor agonist CP-94,253 suppress food intake (e.g. Hewitt et al. 2002; Clifton et al. 2003). Analysis of the pattern of ingestive behaviour suggests that CP-94,253 inhibits feeding by

advancing the 'behavioural satiety sequence' (progression from ingestion, through locomotor activity and grooming, to quiescence) (Lee et al. 2004). The effect of CP-94,253 can be reversed by the selective 5-HT_{1B} receptor antagonist SB-224289 (Lee et al. 2004). These effects are absent in 5-HT_{1B} receptor knockout mice (Lee et al. 2004). These mice are characterized by increased food intake and body weight compared to wild-type mice, further supporting a role of 5-HT_{1B} receptors in the inhibitory regulation of feeding (Bouwknicht et al. 2001). 5-HT_{1B} receptors occur on 5-HTergic terminals (terminal autoreceptors) and on postsynaptic membranes. The effects on feeding are likely to be mediated by postsynaptic receptors, as indicated by the increase in Fos immunoreactivity in the hypothalamus and amygdala following CP-94,253 administration; an action on nerve terminal activity is considered unlikely to result in a change in this index of neuronal activation (Lee et al. 2004).

Although the suppression of feeding by fenfluramine is partly mediated by 5-HT_{1B} receptors (see above), there is also evidence for a role of 5-HT_{2C} receptors in this effect. 5-HT_{2C} receptor knockout mice show reduced sensitivity to fenfluramine (Vickers et al. 1999). Moreover, in intact rats, the hypophagic effect of fenfluramine can be mimicked by the selective 5-HT_{2C} receptor agonist Ro-60-0175, and attenuated by the selective 5-HT_{2C} receptor antagonist SB-242084 (Clifton et al. 2000). Further evidence for a role of 5-HT_{2C} receptors in feeding is provided by the finding that hypophagia induced by 3,4-methylenedioxy-N-methamphetamine (MDMA, 'ecstasy') can be blocked by the selective 5-HT_{2C} receptor antagonist RS-102221 (Conductier et al. 2005).

1.2.4.4. *5-HT and reinforcement*

The putative role of 5-HT in feeding behaviour suggests that behaviour controlled by positive reinforcement might be sensitive to manipulation of the 5-HTergic system, possibly by altering the 'palatability' of food reinforcers (Cooper and Neill 1987).

Wogar et al. (1991) used a quantitative operant paradigm based on Herrnstein's (1970) response strength equation to examine the effect of central 5-HT depletion on reinforcer efficacy. 5-HT depletion reduced the rate of reinforcement needed to maintain response rates at the half-maximum rate, consistent with an enhancement of reinforcer efficacy.

Harrison and Markou (2001) found that systemic treatment with the 5-HT_{1A} receptor agonist 8-OH-DPAT enhanced the reinforcing efficacy of hypothalamic stimulation. This effect was mimicked by direct injection of 8-OH-DPAT into the median (but not the dorsal) raphe nucleus, suggesting that the effect was mediated by 'switching off' the 5-HTergic projection via somatodendritic autoreceptors.

Fletcher et al. (1999) found that 5-HT depletion enhanced responding for a conditioned reinforcer. This suggests that the effect of 5-HT depletion on positive reinforcement is not simply due to an alteration of food palatability.

1.2.4.5. *5-HT and learning and memory*

Central 5-HT depletion has been found to have complex effects on learning and memory. Spatial learning in the Morris water maze has been found to be impaired following 5,7-dihydroxytryptamine-induced 5-HT depletion (Lehman et al. 2000). However, 5-HT depletion has no effect on working memory in delayed non-matching-to-sample tasks (Ruotsalainen et al. 1998).

Graham et al. (1994) found that 5-HT depletion facilitated the learning of a difficult temporal discrimination (discrimination between 200-ms and 800-ms light presentations). It was suggested that this effect might have been due to a reduction of proactive interference between trials. However, a detailed analysis of the effect of 5-HT depletion on performance in delayed matching-to-sample tasks using stimuli of different durations failed to find any effect on proactive interference (Al-Ruwaitea et al. 1997). In contrast to its facilitatory effect on acquisition in temporal discrimination tasks, 5-HT depletion impedes learning in tasks requiring delayed responding (Wogar et al. 1992; Al-Ruwaitea et al. 1997).

The roles of different 5-HT receptor subtypes in learning and memory are complex and, in some cases, controversial. Stimulation of postsynaptic 5-HT_{1A} receptors in the hippocampus by 8-OH-DPAT results in impairment of spatial memory in the water maze, an effect that can be reversed by WAY-100635 (Carli et al. 1995). 8-OH-DPAT injected into the raphe nuclei, thereby presumably suppressing ongoing 5-HTergic activity, has been reported to improve spatial memory (Warburton et al. 1997), an observation that is difficult to reconcile with the deleterious effect of 5-HT depletion on performance in the water maze (see above).

5-HT_{1B} receptor stimulation in the hippocampus induced by

intrahippocampal injection of the selective agonist CP-93,129 has been reported to impair spatial memory (Buhot et al. 1995). Another 5-HT_{1B} receptor agonist, GR-46611, has been reported to impair learning in an autoshaping task, an effect that could be reversed by the selective antagonist GR-127935 (Meneses et al. 1997).

There has been considerable interest in the possible role of 5-HT₄ receptors in learning and memory (see Bockaert et al. 2004). For example, the 5-HT₄ receptor partial agonist RS-67333 has been reported to facilitate object recognition and spatial memory (Lamirault and Simon 2001), whereas olfactory memory may be impaired by the 5-HT₄ receptor antagonist RS-67532 (Marchietti et al. 2000).

Finally, there is increasing evidence for a role of 5-HT₆ receptors in some aspects of learning and memory. For example, 5-HT₆ receptor antagonists have been found to enhance acquisition in an autoshaping task, and to reverse the impairment of learning induced by the cholinergic antagonist scopolamine (Meneses 2001). It has been suggested that 5-HT₆ receptors may regulate cholinergic transmission in some areas of the brain such as the hippocampus, that are believed to be important for learning and memory (Branchek and Blackburn 2000).

1.2.4.6. *5-HT and 'impulsiveness'*

It has long been suspected that dysfunction of 5-HTergic mechanisms is a contributory factor in human "impulsiveness" (Linnoila et al. 1983; Coccaro et al. 1989; Linnoila and Virkkunen 1992; Coscina 1997). A major problem in

this area is the definition of impulsiveness. Clinical definitions generally emphasise “action without due thought”, and often fail to distinguish between aggressive or violent behaviour and other forms of “impulsiveness” (see Ho et al. 1998, 1999; Evenden 1999; Hollander and Rosen 2000). Definitions based on behavioural principles have been proposed by Evenden (1999) and Ho et al. (1999). According to Ho et al. (1999), it is useful to distinguish between two quite different forms of “impulsiveness”: *impulsive action* and *impulsive choice*. The former refers to premature responding in situations where delayed responding is advantageous; the latter refers to selection of small short-term gains in preference to larger delayed gains, and corresponds to the definition of impulsiveness championed by Ainslie (1974). Both forms of impulsiveness are relevant to the topic of this thesis, because both generally entail timing; impulsive action may be revealed by tasks which require the subject to withhold a response for a specified duration (differential-reinforcement-of-low-rates [DRL] schedule: Ferster and Skinner 1957), whereas impulsive choice may be revealed in inter-temporal choice schedules (see below, Section 1.3).

The putative role of 5-HT in timing performance will be reviewed in greater detail in a subsequent section. However, it may be noted at this point that destruction of the 5-HTergic pathways appears to promote both forms of impulsive responding. Thus, central 5-HT depletion impairs performance in delayed response schedules (Wogar et al. 1992; Carli and Samanin 2000; Winstanley et al. 2004), and promotes the selection of smaller immediate rewards in preference to larger delayed rewards (Wogar et al. 1993; Al-Ruwaitea et al. 1999b; Mobini et al. 2000; however, see Winstanley et al. 2004 for contrary results). These results suggest that the 5-HTergic pathways

normally place some form of restraint on impulsive responding. Consistent with this suggestion, the 5-HT releasing agent dexfenfluramine (Poulos et al. 1996) and the 5-HT reuptake inhibitors fluoxetine, citalopram and paroxetine (Wolff and Leander 2002) promoted rats' selection of the larger delayed reward in an inter-temporal choice schedule ('self-controlled choice'). Cherek and Lane (1999) obtained similar results with fenfluramine in a group of human subjects with histories of conduct disorder.

Little is known about the 5-HT receptors involved in impulsiveness. The 5-HT_{1A} receptor agonist 8-OH-DPAT enhanced "impulsive action" in a serial reaction time task (Carli and Samanin 2000), and promoted "impulsive choice" in a delay-discounting task (Winstanley et al. 2005). The highly selective 5-HT_{2A} receptor antagonist MDL-100907 reversed the impairment of DRL performance induced by the *N*-methyl-D-aspartate (NMDA) antagonist dizocilpine, whereas the 5-HT_{2C} receptor antagonist SB-242084 tended to exacerbate the effect (Higgins et al. 2003).

1.3. TIMING PARADIGMS

1.3.1. Introduction: timing paradigms and schedules of reinforcement

A reinforcement schedule is a rule that describes how some readily measured behaviour (such as pressing the lever, for a rat, or pecking at a coloured light, for a pigeon) affects the delivery of food or some other positively or negatively valued event (Ferster and Skinner 1957; Staddon et al. 1991). The principal schedules of positive reinforcement first defined by Skinner (1938), and

subsequently studied in great detail by Ferster and Skinner (1957), are *fixed* and *variable interval* (FI and VI), and *fixed* and *variable ratio* (FR and VR) schedules. In each case, a response is required for the delivery of a reinforcer (usually a small amount of food). On a *fixed-interval* schedule, the response is effective only after a fixed time period, *I*, has elapsed since the preceding reinforcer delivery. On a *fixed-ratio* schedule, only the *N*th response after reinforcer delivery is effective. On *variable-interval* and *variable-ratio* schedules, *I* and *N* vary unpredictably from one reinforcer delivery to the next. After an initial learning period these four schedules produce characteristic, regular patterns of behaviour in almost all mammals and birds exposed to them (Staddon et al. 1991). Of these four basic types of schedule, only fixed-interval schedules specify a regular and predicable temporal relation between successive reinforcer deliveries. Nevertheless, there is evidence that the temporal distribution of reinforcer deliveries is an important determinant of performance even in the case of schedules where no explicit temporal contingency is specified (Neuringer and Schneider 1969).

Humans and animals can readily learn to anticipate the time when a reinforcing event will occur. This process is termed *interval timing*. It is typically studied in well-trained animals under steady-state conditions. A large number of reinforcement schedules have been devised to study interval timing behaviour in animals. Killeen and Fetterman (1988) and Killeen et al. (1997) developed a taxonomy of timing schedules based on the relationship between the animal's timing response and the interval being timed. According to this taxonomy, the three main classes of timing schedule are (i) *retrospective* timing schedules, in which the subject is trained to emit discriminative

responses depending upon the duration of an interval which has already elapsed when the response is made; (ii) *immediate* timing schedules, in which the subject's behaviour comes under the control of time during an ongoing interval and (iii) *prospective* timing schedules, in which the animal is trained to emit discriminative responses on the basis of intervals which follow the responses. The aim of this section is to offer a relatively detailed description of each of these three paradigms.

1.3.2. **Retrospective timing schedules**

1.3.2.1. *Conditional temporal discrimination tasks*

In this type of task, the subject is trained to emit one of two mutually exclusive responses following the offset of a signal, the two responses being differentially reinforced depending upon the duration of the signal (see Killeen and Fetterman 1988; Killeen *et al.* 1997). The conventional performance measure is the percentage of correct responses (i.e. the percentage of trials in which a reinforcer is earned).

1.3.2.2. *Interval bisection task*

In this task (Catania 1970; Church and Deluty 1977), the subject is first trained to discriminate two durations ("short" and "long") in a discrete-trials conditional discrimination schedule. When accurate performance has been attained, probe trials, in which stimuli of intermediate duration are presented,

are introduced into each session. In the case of each duration, the percentage of occasions on which the subject responds on the lever appropriate to the long stimulus (%L) is recorded. %L increases as function of stimulus duration; this function approximates closely to a sigmoid logistic curve:

$$\%L = \frac{100}{1 + \left[\frac{t}{T_{50}} \right]^{\varepsilon}}$$

where t is stimulus duration, T_{50} is a location parameter, and ε a slope parameter (Killeen et al. 1997; Ho et al. 2002). The logistic relation between %L and stimulus duration provides the basis for the estimation of two basic indices of interval timing behaviour (Killeen et al. 1997). (i) The measure of central tendency is the bisection point (T_{50} : the duration corresponding to %L = 50), which occurs at about the geometric mean of the two standard durations. (ii) The Weber fraction, a measure of the precision of temporal discrimination, may be computed from the ratio of the limen (half the difference between the durations corresponding to %L = 25 and %L = 75) to the bisection point (Church and Deluty 1977; Ho et al. 2002).

1.3.2.3. *Discrete-trials psychophysical procedure*

In this task (Body et al. 2002a), each session consists of a number of trials, successive trials being initiated at regular intervals. Each trial starts with the illumination of a lamp above the central reinforcer recess. After a predetermined interval has elapsed, two levers are inserted into the operant chamber. A single response on either lever results in withdrawal of both levers and extinguishing of the light; the chamber remains in darkness until the start

of the next trial. A response on one lever (A) is reinforced if the light has been presented for a shorter time than some specified duration, whereas a response on the other lever (B) is reinforced if the light has been presented for longer than the specified duration. In Body et al.'s (2002a) schedule, the trials were 50 s in duration and in each trial the levers were inserted into the chamber at one of the following "entry points" following the start of the trial: 2.5, 7.5, 12.5, 17.5, 22.5, 27.5, 32.5, 37.5, 42.5, or 47.5-sec. A response on A was reinforced if insertion took place at any of the first five entry points (i.e., less than 25 s after trial onset), whereas a response on B was reinforced if lever insertion took place at any of the last five entry points (i.e., more than 25 s after trial onset). Performance on this schedule can be characterized by the same logistic psychometric function as that which describes interval bisection performance (Body et al. 2002a).

1.3.2.4 *Temporal generalization*

In a temporal generalization procedure, a stimulus of some duration is presented; if it is of one particular duration, a response is reinforced (Church and Gibbon 1982). The performance measure is the relationship between probability of responding and stimulus duration. Typically a bell-shaped generalization curve is generated, in which response probability is greatest near the reinforced duration, with approximately symmetrically decreasing probabilities at shorter and longer durations (Church and Gibbon 1982; Wearden 1992; Wearden and Grindrod 2003).

1.3.3. **Immediate timing schedules**

1.3.3.1. *Fixed-interval peak procedure*

The peak procedure was devised by Catania (1970) to study temporal control under fixed-interval schedules. In a fixed-interval schedule food delivery follows the first response emitted after a specified and constant time period has elapsed since the previous reinforcer (Ferster and Skinner 1957; see above). In the peak procedure, the interval is timed from the onset of a trial, usually signalled by insertion of the lever into the chamber, and successive trials are separated by an inter-trial interval. The schedule consists of two types of trial. In “standard” trials, reinforcement is provided for the first response after the fixed interval has elapsed; in “empty” or “probe” trials, the reinforcer is omitted, and the lever remains in the chamber for a long period (usually three or four times longer than the fixed-interval duration) (see Staddon and Cerutti 2003). Behaviour in the probe trials consists of progressively increasing response rate up to the end of the criterion interval, followed by a declining response rate (see Hinton and Meck 1997). The bell-shaped function obtained from this procedure may be used to derive several behavioural indices. The *peak rate* is the highest response rate achieved during the trial, and the *peak time* is the elapsed time within the trial at which the peak rate occurs; the *spread time* may be defined as the interval between the point in time when response rate reaches 70% of the peak rate, until it first falls below that level,

and the *Weber fraction* may be calculated as the ratio of the spread time to the peak time (Church et al. 1991). Numerous studies have shown that the peak time occurs close to the time at which reinforcement occurs in the standard trials, and that the Weber fraction remains roughly constant across a broad range of criterion intervals (see Hinton and Meck 1997; Lejeune et al. 1998).

1.3.3.2. *Mixed fixed-interval schedules*

Another immediate timing schedule devised by Catania (1970) is a mixed schedule consisting of two fixed-interval components. In this schedule, the subject is trained in discrete trials in which a reinforcer is available either t seconds or $t \times n$ seconds after the onset of the trial. Typical performance consists of an initial rise in response rate which achieves a peak approximately t seconds after trial onset, followed by a decline in response rate, and then a secondary rise in response rate that reaches its peak about $t \times n$ seconds after trial onset. Leake and Gibbon (1995) and Whitaker et al. (2003) examined the performance of rats under this schedule using a range of values of t and n . In general, clear separation of the two peaks can only be discerned when $n > 3$; this appears to be the case irrespective of the value of t (Whitaker et al. 2003).

1.3.3.3. *'Tri-peak' procedure*

Another recently developed variant of the fixed-interval peak procedure is the "tri-peak" procedure (Paule et al. 1999). This schedule requires subjects to track three target durations presented sequentially within a single trial. This is

accomplished by pairing a different response lever with each target duration (e.g., 10, 30 and 90-s). Rats tend to produce uneven response rates on the three operanda during the course of the trials, with peaks corresponding to the scheduled times of reinforcer delivery on each lever. An advantage of this method is that it allows measurement of the “start time”, “stop time”, “peak time” and “spread time” for each of the three durations, as well as correlations among these measures within a single session (Church et al. 1994).

1.3.3.4. *‘Gap’ procedure*

This is a variant of the peak procedure designed to examine the effect of interrupting an ongoing timing task (Roberts 1981). Animals are first trained under a fixed-interval procedure. Then, in probe trials interspersed among the standard peak interval trials, a “gap” is introduced; for example, if the trials are signalled by a continuously present tone, the tone may be discontinued for a brief period, and the duration of this interruption is added to the duration of the interval. It has been found that the effect of the gap depends on its location within the trial. If the gap is presented early in the trial, it has little effect on the time of peak responding; however gaps presented later in the interval tend to induce a delay of the peak time. These effects have been interpreted as indicating a “stopping and re-starting” of an internal timing process in the former case, and the “resetting” of the process in the latter (Matell and Meck 1999; Buhusi and Meck 2000).

1.3.3.5. *Free-operant psychophysical procedure*

The free-operant psychophysical procedure was devised by Stubbs (1976, 1980). In this schedule, each experimental session consist of a series of trials in which reinforcement is provided, under a variable-interval schedule, for responding on one of two continuously available operanda. Reinforcement availability is allocated to operandum A during the first half, and to operandum B during the second half of each trial. The typical pattern of responding on this schedule consists of an increasing response rate on operandum B and a concomitantly declining response rate on operandum A during the course of the trial. This is reflected in an increasing relative response rate on operandum B (*i.e.* response rate on operandum B divided by the combined response rate on both operanda), which passes the “indifference point” T_{50} (50% responding on operandum B) approximately midway through the trial, when reinforcer availability is transferred from operandum A to operandum B. The relationship between relative response rate and time, measured from the onset of the trial, is well described by the same logistic function that has been found to define the psychometric curve in retrospective timing tasks (Stubbs 1979, 1980; Bizo and White 1994; Killeen et al. 1997; Chiang et al. 1998).

Chiang et al. (1998, 1999, 2000a, 2000b) introduced a modification to the free-operant psychophysical procedure. In Stubbs’ (1976) original schedule, the subject is able to switch back and forth between the two operanda throughout the trial (“unconstrained switching”). In contrast, in Chiang et al.’s (1998) modified schedule, the first response to occur on lever B results in withdrawal of lever A. This has the effect of restricting switching to one switch per trial (“constrained switching”). The resulting psychometric curves tend to

be steeper than those obtained in the conventional “unconstrained-switching” task, although the locus of T_{50} is not greatly affected by the imposition of the constraint (Chiang et al. 1998, 1999, 2000a, 2000b).

1.3.3.6. *Differential-reinforcement-of-low-response rate (DRL)*

In this schedule, also known as the “inter-response-time-greater-than- t (IRT> t)” schedule, reinforcer delivery follows every response that is separated from the previous response by an interval of at least t s (Ferster and Skinner 1957; Zeiler 1977). Steady-state behaviour on this schedule consists of low overall responses rates, with long interresponse times interspersed by brief bursts of responding. The long interresponse times have a mode that approximates to the criterion interresponse time (t); there is, however, a tendency for short criterion interresponse times to be overestimated and long ones to be underestimated, resulting in a linear relation between criterion and actual interresponse times that has a slope considerably less than one. The variation of interresponse times about the modal value is approximately normal in form, and the ratio of the standard deviation to the modal interresponse time has often been used as an expression of the Weber fraction (Platt 1979)

1.3.4. **Prospective timing schedules**

Prospective timing schedules entail the control of behaviour by events that follow the response by a specified time interval. In most instances the controlling event is reinforcement, and thus tasks of this type frequently take

the form of intertemporal choice schedules, in which the subject chooses between reinforcers differing in magnitude and delay (see Ho et al. 2002).

1.3.4.1. *Adjusting delay schedule*

In this schedule (Mazur 1987), the subject undergoes a series of discrete trials in which it chooses between a smaller reinforcer, A, delivered after a short delay, d_A , and a larger reinforcer, B, delivered after a variable delay, d_B . Sessions are divided into blocks of trials, and the value of d_B is varied in successive blocks as a function of the subject's choices in the previous block. If the subject displays a preference for A, d_B is reduced in the following block; conversely, if the subject shows a preference for B, d_B is increased in the following block. Mazur (1987) showed that behaviour on this schedule is characterized by fluctuations in the obtained value of d_B , which eventually stabilize; the stable value of d_B , which is taken as a measure of the "indifference point", is sensitive to the relative sizes of the reinforcers and the delay imposed on the smaller reinforcer (d_A) (Mazur 1987; Ho et al. 1999).

1.3.4.2. *Time-left procedure*

This is a two-link concurrent chain schedule (Autor 1960), in which reinforcement is provided for responding on two operanda, A and B, in a series of trials each lasting T sec. At the start of the trial, both operanda are available (initial link). At a randomly determined time point, t seconds after the start of the trial, the next response on either operandum results in entry into one of the two mutually exclusive terminal links, each of which terminates in reinforcer

delivery. A response on operandum A initiates a fixed delay (d_A) followed by reinforcement, whereas a response on operandum B initiates a variable delay (d_B) followed by reinforcement at the end of the trial. The length of d_B is thus determined by the value of t , i.e., by the time elapsed since the start of trial (Gibbon and Church 1981; Gibbon and Fairhurst 1994). Performance on this schedule consists of increasing preference for B as a function of time from the start of the trial (e.g. Al-Ruwaitea et al. 1999a). Preference can generally be described by a sigmoid (approximately logistic) psychometric function, and the Weber fraction is approximately constant across a range of values of T (Gibbon and Fairhurst 1994).

1.3.4.3. *Progressive delay schedule*

Evenden and Ryan (1996) described a procedure in which rats were trained to make repeated choices between a small immediate reinforcer, A, and a larger delayed reinforcer, B, the delay to the larger reinforcer being increased progressively in successive blocks of trials within a session. This method was modified by Kheramin et al. (2002). In Kheramin et al.'s schedule, a short delay, d_A , was imposed on the smaller reinforcer; the delay to the larger reinforcer (d_B) was the same as d_A at the start of the session, and was incremented in successive blocks of trials according to the formula $d_B = d_A \times (1.75)^{n-1}$, where n is the ordinal position of the block of trials within the session. Kheramin et al. (2002) found that percent choice of the larger reinforcer decreased asymptotically towards zero as a function of d_B . When the 'indifference delay to B' (the value of d_B corresponding to 50% choice of B)

was plotted against the delay imposed on A (d_A), a linear relation was revealed, consistent with the model of intertemporal choice proposed by Ho et al. (1999).

1.4. THEORIES OF INTERVAL TIMING

A number of theoretical models of interval timing behaviour have been proposed. In this section, only those models that have been applied to the behaviour of animals in the timing paradigms described above will be reviewed. The oldest and most widely studied of these models is Scalar Expectancy Theory (SET); accordingly, this theory will receive greater attention in the following review than other, newer theories that have not yet been subjected to extensive experimental examination.

1.4.1. Scalar Expectancy Theory (SET)

The Scalar Expectancy Theory (SET) was conceived and developed by John Gibbon (Gibbon 1977, 1981; Gibbon et al. 1984; Malapani and Fairhurst 2002) and remains today the most prominent of the theoretical accounts of timing in animals and humans. It consists of a mechanistic interpretation of timing behaviour based on information processing principles, which account for the principal phenomena of timing behaviour outlined in the previous section.

The central principle of SET is that interval timing behaviour is *scalar*. This means that the accuracy (or rather its converse, the variability) of timing is deemed to be proportional to the target interval being timed, in accordance to Weber's law. This implies that the *relative* accuracy of timing, expressed as the Weber fraction, should be constant. For example, if a subject trained under an

inter-response time schedule to emit responses at 15-s intervals, generates an interresponse time distribution with a standard deviation of 5 s (Weber fraction, expressed as the coefficient of variation, $= 5/15 = 0.33$), then the same subject trained to emit responses at 30-s intervals should display a standard deviation of 10 s (Weber fraction $= 10/30 = 0.33$).

An extension of this principle is the notion of superposability of psychometric functions. For example, if the logistic psychometric functions derived from performance on two interval bisection schedules are re-scaled in such a way that time (t) is expressed as a fraction of the indifference point (T_{50}), the two sigmoid curves should become identical (Gibbon 1977, 1991).

According to SET, organisms possess an endogenous ‘internal clock’ that enables them to perform interval timing tasks. This clock is composed of several components, each of which generates a certain amount of variance in the organism’s timing behaviour. These various sources of variance are random, and are not in themselves scalar. However, the overall variability of timing which, according to the theory, is derived from a multiplicative combination of the various sources of variance, is scalar (see Gibbon 1991).

The structure of the hypothetical internal clock posited by SET is shown in Figure 1.2. The operation of the clock is divided into three stages, which are usually considered sequentially. The first stage involves a pacemaker connected to an accumulator or counter, which receives the pacemaker pulses via a switch. The switch controls when the flow of pulses into the accumulator is started and stopped. The accumulator stores the total number of pulses which represent the amount of time passed. The contents of the accumulator can be copied to a short term memory (STM) or working memory. In some versions of

SET, the accumulator and STM are regarded as one entity (Wearden et al. 1999). Information is transferred to another memory store, long term memory

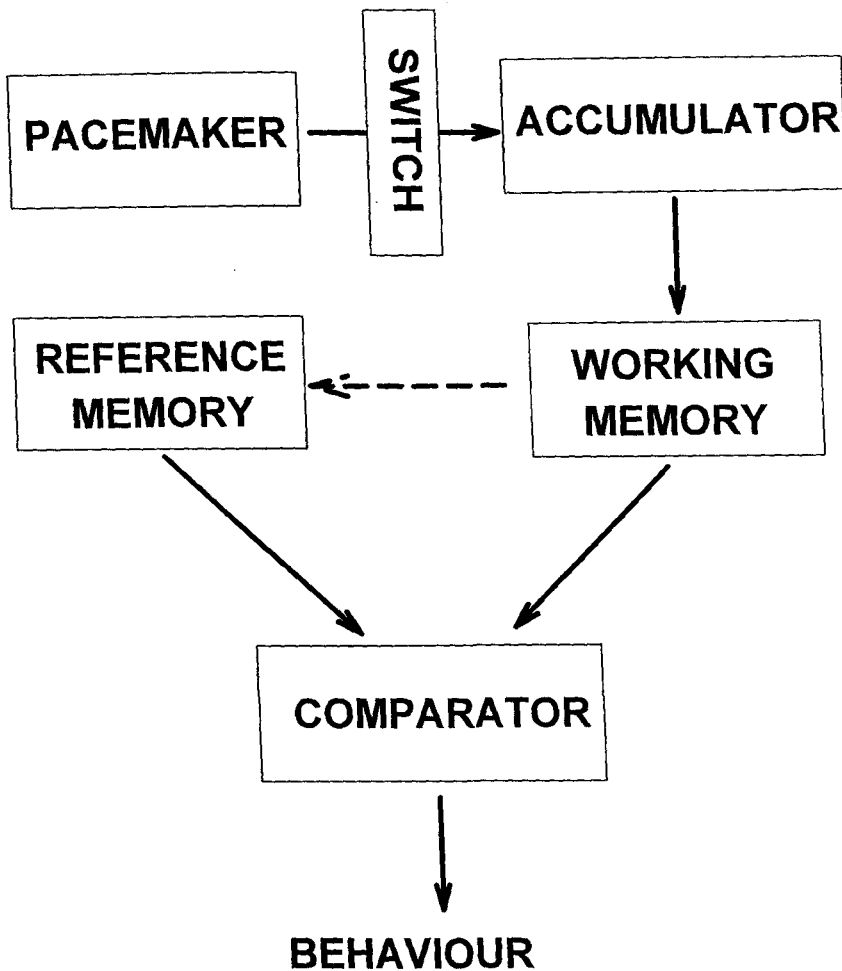


Figure 1.2. Model of the hypothetical internal clock proposed by Scalar Expectancy Theory. According to the theory, an endogenous pacemaker generates pulses at a constant mean rate, and the accumulator counts the pulses. In timing an interval, the organism is supposed to compare the current number of pulses (in its working memory) to a sample of previously recorded numbers of pulses (in its reference memory). If the current number is approximately equal to the sample from reference memory, the organism emits the appropriate timing response.

(LTM) or reference memory. LTM is able to retain a record of many instances of the expected time of some relevant event (e.g. reinforcement time). Finally, there is a comparator, or decision-maker, which assesses the similarity between the contents of STM and LTM, and if the values are sufficiently similar a response is made.

In Gibbon's (1977, 1991) original version of SET, there was no attempt to identify the components of the hypothetical internal clock with actual anatomical structures or physiological processes. The model was conceived as a 'black box' whose performance closely approximated the behaviour of real organisms. However, the discovery that the effects of drugs on interval timing behaviour could be characterized as changes in the functioning of the various components of the hypothetical clock (Meck 1986; 1996) led some proponents of SET to identify these components with particular pathways or nuclei in the brain (e.g. Gibbon et al. 1997a; Hinton and Meck 1997, 2004; Matell and Meck 2000, 2004; see below).

1.4.2. Behavioural Theory of Timing (BeT)

The Behavioural Theory of Timing (BeT: Killeen and Fetterman 1988; Killeen et al. 1997) is another pacemaker-based theory. Like SET, BeT assumes an endogenous pacemaker that emits pulses at more-or-less constant rate. However, unlike the essentially cognitive mechanisms proposed by SET, BeT assumes that pacemaker's role is to drive the organism through a series of behavioural states (Ho et al. 2002). Each state is associated with a different class of behaviour, and these behaviours serve as discriminative stimuli that set

the occasion for appropriate operant responses.

BeT is based on the following premises (Killeen and Fetterman 1988):

- (i) Stimuli that signal reward engender responses. These responses are called *adjunctive*, because they can be elicited or emitted (Killeen & Fetterman 1988; Morgan et al. 1993).
- (ii) Transitions between adjunctive behaviours are caused by pulses from the pacemaker. The pacemaker in this model is started at the onset of the interval being timed, and it drives the animal into different behavioural states with each pulse.
- (iii) During the learning of a timing task, operant responses come to be associated with adjunctive 'behavioural states' by Pavlovian conditioning. Thus, after extensive training, the animal learns that when a particular state in the sequence has been reached, an operant response will result in reinforcement.
- (iv) Pacemaker speed is proportional to reinforcement density. This is a unique feature of BeT (Morgan et al. 1993; see below).

Killeen and Fetterman (1988) argue that cognitive processes such as memory and decision processes, which play a central role in SET, are not really necessary to account for interval timing behaviour. Instead of the complex cognitive apparatus posited by SET, BeT posit only one hypothetical construct, the endogenous pacemaker. In order to account for the scalar property of timing, BeT assumes that the accuracy of timing is determined by the rate of the pacemaker, which is in turn determined by the rate of reinforcement. In other words, timing an interval is likely to be more accurate if the inter-pulse

interval is short (analogous to the ticking of a wrist-watch) than if it is long (analogous to the ticking of a grandfather clock). The significance of the postulated relation between reinforcement rate and pacemaker speed can be appreciated from the example of fixed-interval peak performance. In a peak fixed-interval 30-s schedule the reinforcement rate is twice as high as in a peak fixed-interval 60-s schedule; accuracy should therefore be greater in the former case than in the latter, leading to approximately equivalent *relative* accuracy in the two cases (i.e. conformity to Weber's law).

It follows from this argument that if reinforcement rate is altered without changing the length of the interval being timed, a reduction of the Weber fraction should be observed. This prediction was confirmed by Bizo and White (1994) in the case of the free-operant psychophysical procedure; doubling the rate of 'background' reinforcement provided throughout the trials by a variable-interval schedule did not alter T_{50} but substantially reduced the Weber fraction.

1.4.3. **Learning to Time (LeT) theory**

This theory is a refinement of BeT, designed to account not only for steady-state timing behaviour, but also for the acquisition and extinction of interval timing (Machado 1997; Machado and Guilhardi 2000). Like BeT, each time marker (e.g. reinforcer delivery) is assumed to activate a series of behavioural states. These states include elicited, interim and terminal classes of behaviour (Staddon and Simmelhag 1971; Staddon 1977; Killeen and Fetterman 1988). Elicited behaviours, for example retrieving and consuming food in the food

hopper by a rat, may occur immediately after reinforcer delivery. Interim behaviours, for example grooming or exploration, may occur in the middle of interval, when getting food is not probable. Terminal behaviours, for example approach to the food hopper, can be seen in the final segment of the interval. At the onset of the interval only the first state is active, but as time goes on the other states become activated sequentially. Like BeT, LeT assumes that the operant response becomes coupled to the behavioural states by a process of Pavlovian conditioning, the associative strength depending on the proximity of the state to reinforcer delivery. If a state is active during extinction, it loses its coupling and eventually may not support the operant response. If a state is active during reinforcement, its coupling will be increased and may therefore sustain the response.

The mathematical aspects of the model are beyond the scope of this overview. In essence, however, it is assumed that by adding the activation strength of the behavioural states, the strength of each operant response can be obtained (Machado 1997; Machado & Guilhardi 2000). A crucial difference between BeT and LeT is that in BeT, discrete behavioural states occur in sequence, only one of which becomes coupled to the operant response; LeT, in contrast, proposes a graded strength of association of the operant response with all the behavioural states that make up the sequence (Machado 1997).

LeT is able to account for all the principal phenomena of interval timing behaviour, including its scalar property. However, Machado (1997) has drawn attention to some quantitative discrepancies between predicted and actual behaviour. These include modest discrepancies between the theoretical and empirical peak times in the fixed-interval peak procedure, and indifference

points (T_{50}) in the interval bisection procedure. Machado (1997) acknowledged that these discrepancies can only be accounted for by arbitrary post hoc assumptions of delayed onset of timing. It should be noted, however, that other timing models, including SET, are forced to make similar arbitrary assumptions (e.g. ‘attentional latency’; see Hinton and Meck 1997).

Machado and Guilhardi (2000) have pointed out that a crucial difference between SET on the one hand, and BeT and LeT on the other, is the essential role of reinforcement rate in determining pacemaker rate in the behavioural theories. In contrast, reinforcement plays no role in the cognitive mechanisms of SET, other than in sustaining the index response and providing markers for timing. SET therefore has difficulty explaining the findings of Bizo and White (1995) and Machado and Guilhardi (2000), that the value of T_{50} in the free-operant psychophysical procedure can be manipulated by providing different ‘background’ variable-interval schedules in the first and second halves of the trials. Thus, if the schedule provided for responding on lever A in the first half of the trial offers a higher rate of reinforcement than the schedule provided for responding on lever B in the latter half of the trial, T_{50} is reduced (i.e. the psychometric curve is displaced to the left); conversely, if the schedule operating in the second half is richer than the one operating in the first half, T_{50} is increased (i.e. the psychometric curve is displaced to the right) (Bizo and White 1995; Machado and Guilhardi 2000).

1.4.4. Multiple Time Scales Theory

Multiple Time Scales Theory (MTS: Staddon and Higa 1996; Staddon et al.

1999; 2002) is the only major timing theory at the present time that does not posit a pacemaker-based internal clock. Like the theories outlined above, MTS assumes that interval timing behaviour is derived from a unitary endogenous timing process; however, unlike pacemaker-based theories, MTS does not assume that this process consists of repetitive emission of discrete pulses. Rather, the 'clock' proposed by MTS is based on the phenomenon of habituation (Staddon and Higa 1996).

Staddon and Higa (1996) summarize habituation as the waning of a response to a stimulus, as the stimulus is repeatedly presented. Two important characteristics of habituation are *stimulus specificity* and *rate sensitivity*. Stimulus specificity means that when habituation has occurred for one stimulus, it does not extend to others. Rate sensitivity can be seen when the time between successive presentations of the stimulus (inter-stimulus interval [ISI]) is varied. Habituation is more rapid when ISIs are short than when they are long; moreover, recovery from habituation occurs more rapidly following short ISIs than following long ISIs (Staddon and Higa 1996).

According to MTS, habituation arises from a cascade of 'habituation units', each of which entails an exponential time-dependent decay of the memory trace for the stimulus. This is illustrated in Figure 1.3. During successive presentations of a stimulus, the response to each presentation is determined by a simple subtractive relation:

$$\text{Response} = (\text{direct effect of the stimulus}) - (\text{remembered effect})$$

Because the remembered effect decays exponentially, this simple relation can

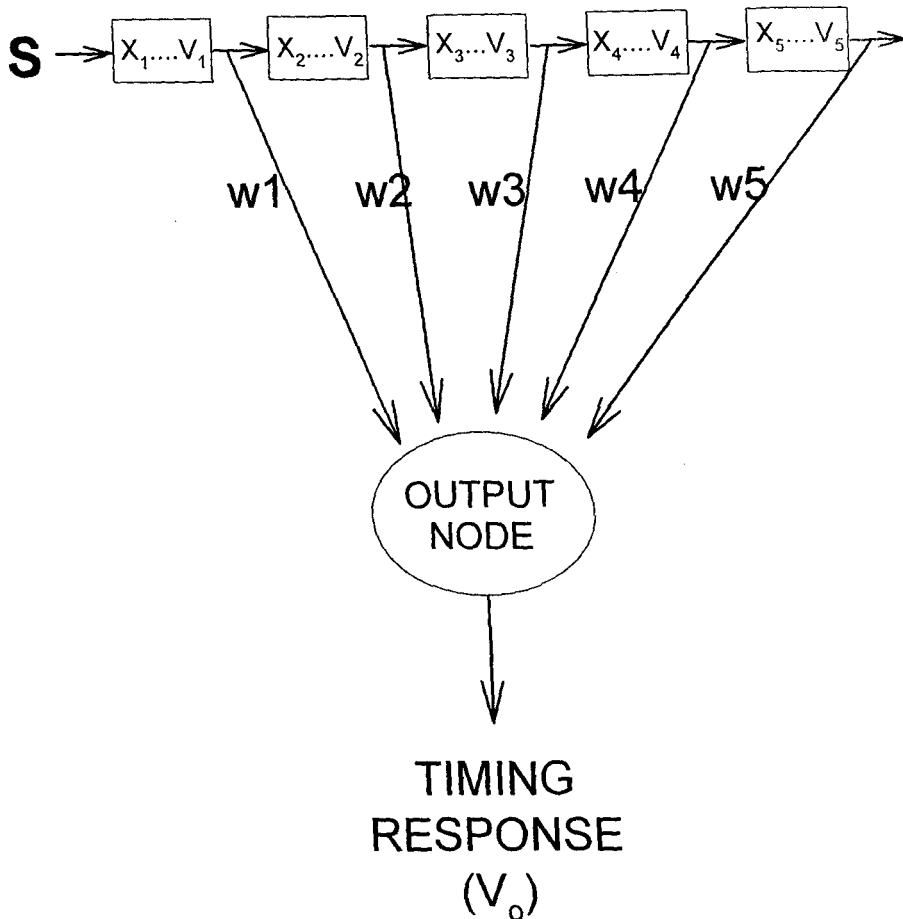


Figure 1.3. ‘Cascade’ of multiple habituation units proposed by the Multiple Time Scales theory of interval timing. Each integrator (represented by the boxes) receives an input (X), and generates an output (V) according to an exponential decay (habituation) function. The stimulus (S) activates the first integrator, whose output (V_1) provides the input (X_2) for the second integrator, and so on. The output from each integrator also provides a weighted connection to an ‘output node’, which generates the overall timing output (V_o) (after Higa and Staddon 1997).

account for the rate-sensitivity phenomenon: the 'remembered effect' is greater with short ISIs than with long ISIs, and therefore the response tends to become smaller (i.e. there is greater habituation) with short ISIs (Staddon and Higa 1996).

Staddon et al. (1999) proposed that MTS can account for interval timing behaviour. In brief, it is proposed that the memory-trace constitutes the 'clock' for steady-state interval timing (Staddon et al. 2002). As in SET, the reinforcer serves as a time-marker, the memory-trace of which decays according to the principles of habituation (see above). In fact, in MTS the clock is just the memory for the time-marker, because in this theory the process of starting and stopping the clock (timing function) and the clock are not separable (Staddon et al. 2002). Operant responses become conditioned to particular strengths of the memory trace. For example, in the case of the fixed-interval peak procedure, the animal learns that responding is most likely to result in reinforcement when the memory-trace of the previous reinforcer has decayed to a particular level.

MTS has been applied mainly to fixed-interval schedules and response-initiated delay schedules (in which the fixed interval preceding each reinforcer is initiated by the first response after the previous reinforcer: Higa and Staddon 1997; Staddon et al. 1999). Proponents of SET have criticized MTS because it fails to address questions about the variability of timing, and in particular it fails to predict the constancy of relative variability (Weber's law) (Gallistel 1999; Gibbon 1999). In response to these criticisms Staddon et al. (1999) argued that scalar invariance is at best only approximately true (see also Grondin 2001), and that MTS is able to accommodate approximate conformity

to Weber's law.

1.4.5. Coincidence Detection Theory (Striatal Beat Frequency Model)

The theories reviewed above attempt to provide 'working models' of interval timing performance, without making specific assumptions about the brain mechanisms underlying timing behaviour. Meck (1986, 1996) and Hinton and Meck (1997) attempted to relate the components of SET to particular brain structures. According to these authors, the nigrostriatal dopaminergic pathway is the neural substrate of the internal clock, the substantia nigra representing the pacemaker, and the corpus striatum the accumulator. Evidence cited in support of this proposal included the leftward displacement of the peak interval function by the dopamine-releasing agent amphetamine (attributed to a reduction of the period of the pacemaker) and the rightward shift of the function produced by D₂ dopamine receptor antagonists (attributed to slowing of the pacemaker). According to Hinton and Meck (1997) 5-hydroxytryptaminergic (5-HTergic) input to the striatum may serve to oppose the dopaminergic influence on 'clock speed'. The memory component of SET has been identified with cholinergic mechanisms in the hippocampus and neocortex (Olton et al. 1987, 1988).

A weakness of this view of the brain mechanisms underlying interval timing was pointed out by Matell and Meck (2000). Interval timing typically entails adaptation of behaviour to events taking place in the range of seconds, minutes, or even hours (Gibbon et al. 1997a), whereas there are no known neural events within the basal ganglia that occur on such a long time scale.

Matell and Meck (2000) proposed that a solution to this problem might be found in the idea of 'coincidence detection'; the simultaneous occurrence of neural signals that occur regularly but with different frequencies could have a period that is very much longer than the period of any one of the individual signals (Miall 1989, 1996). Adapting this principle to interval timing, Matell and Meck (2000) suggested that striatal neurones receive multiple oscillatory inputs from the cortex, discharging in response to the coincidence of a finite number of inputs. These events were proposed to constitute the neural bases of SET's pacemaker. This proposal has been greatly elaborated by Matell and Meck (2004); their revised model is illustrated in Figure 1.4.

The revised Striatal Beat Frequency (SBF) model (Matell and Meck 2004) is based on the striatum's position within a cortico-striato-thalamo-cortical loop. Spiny neurones of the striatum are assumed to function as coincidence detectors of cortical and thalamic input. The cortical and thalamic oscillations constitute the pacemaker pulses, while the spiny neurones function as the accumulator. Memory storage is supposed to be achieved by setting the activity of an ensemble of cortical neurones in such a way that the striatal spiny neurones fire at a designated time. Dopaminergic input to the striatum is no longer assumed to play a central role in the generation of pacemaker pulses, although it is assumed to contribute to modulating pacemaker speed. The SBF model has been tested in computer simulations of timing behaviour, and has proved capable of generating an output that resembles peak fixed-interval schedule performance with criterion intervals within the range of seconds or minutes (Matell and Meck 2004). However, as yet, there is no direct evidence that the neural events postulated by the SBF model actually occur

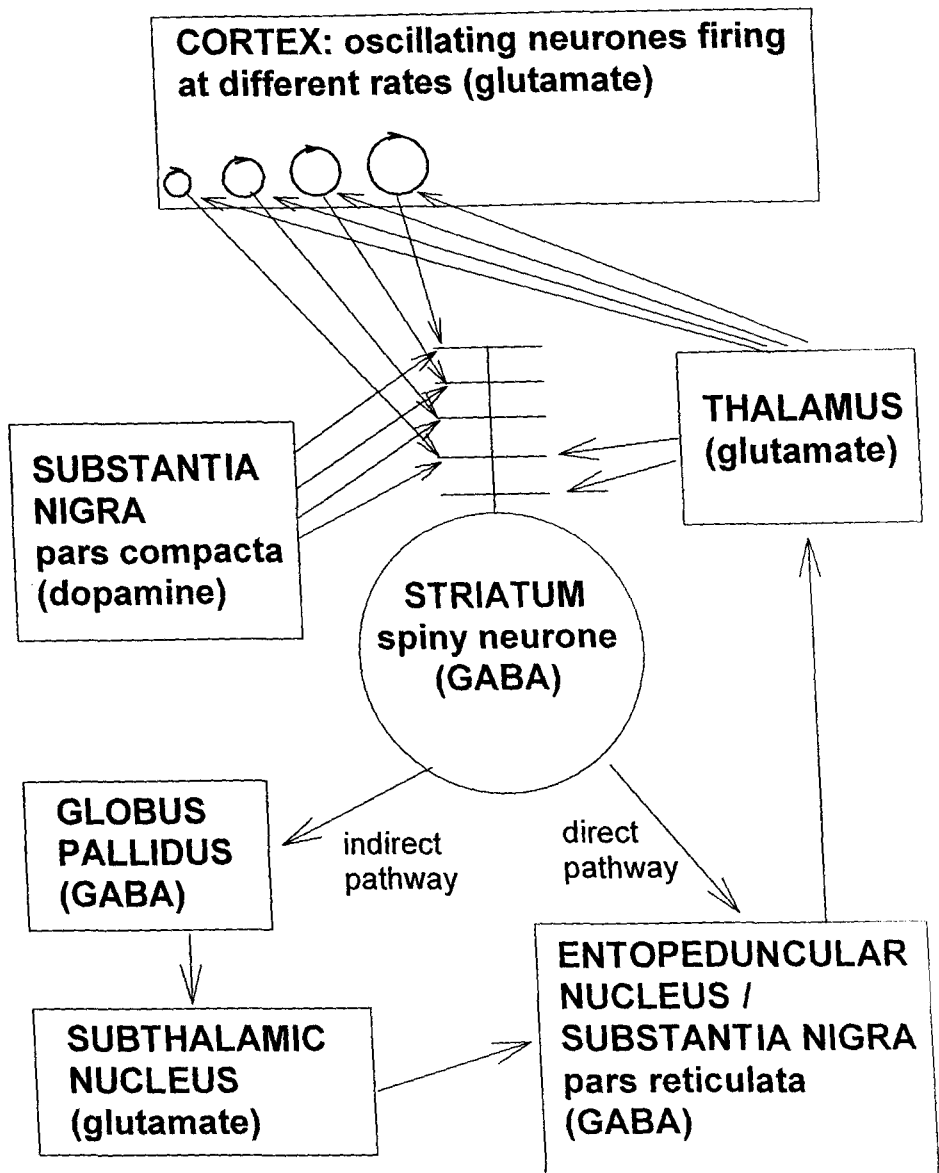


Fig. 1.4. Neural circuitry proposed to account for interval timing by the modified Striatal Beat Frequency theory (Matell and Meck 2000, 2004). It is proposed that during interval timing, activity in the cortex is synchronized by the onset of a stimulus, after which the cortical activity continues with a variety of oscillatory periods. The coincident activity of a subset of these neurones is detected by striatal spiny neurones. The output of these neurones is integrated by the basal ganglia output nuclei (entopeduncular nucleus and substantia nigra pars reticulata), and relayed to the thalamus for behavioural expression. Dopaminergic input from the substantia nigra pars compacta and 5-HTergic input from the dorsal raphe nucleus (not shown) provide ‘tuning’ to the system, and the thalamus provides feedback via its inputs to the cortex and the striatum (after Matell and Meck 2000).

during interval timing behaviour.

Like all the other models of timing behaviour discussed in this section, the SBF model assumes a unitary timing mechanism that underlies all forms of interval timing behaviour. This leads to the prediction, which will be addressed by the experiments described in later chapters, that pharmacological manipulation of the putative neural substrate of timing should give rise to equivalent effects on timing performance in different types of timing schedule.

1.5. BEHAVIOURAL PHARMACOLOGY OF TIMING

Behavioural pharmacology has been described as the synthesis of the experimental analysis of behaviour and pharmacology (Odum 2002), and is focused on investigating the behavioural effects of drugs by manipulating the environment and pharmacology in different ways. One of the most important variables in the experimental analysis of behaviour is time. Different drugs have been used in different categories of temporally-based schedules; and different authors in recent years have been trying to investigate pharmacological and environmental underpinnings of timing. The aim of this section is to summarize research in this area. In recent years increasing attention has been paid to the neural mechanisms underlying interval timing behaviour. Three neurotransmitter systems have been mainly implicated in these mechanisms: the dopaminergic, cholinergic and 5-HTergic systems. These are reviewed in the following three sections.

1.5.1. Dopaminergic mechanisms and timing

The prototypical timing schedule is the fixed-interval schedule, in which reinforcement is provided following the first response to be emitted after a designated interval since the previous reinforcer delivery (Ferster and Skinner 1957; see above). Skinner (1938) was the first to report the sensitivity of fixed-interval schedule performance to the catecholamine releasing agent amphetamine. Dews (1958) carried out the first quantitative analysis of amphetamine's effect on fixed-interval performance, and formulated the descriptive principle of 'rate-dependency'. According to the rate-dependency principle, the effect of a drug on response rate depends on the baseline response rates; low response rates tend to be increased, and high rates to be suppressed. This principle is consistent with amphetamine's tendency to facilitate responding in the early part of a fixed interval (when baseline response rate is low), and to suppress responding later in the interval (when baseline response rate is high). Although Dews' (1958) early observations were based on fixed-interval schedule performance, the rate-dependency principle does not attribute the effects of drugs to an interaction with timing processes; indeed Sanger and Blackman (1976) Dews and Wenger (1977) reviewed a body of evidence suggesting that amphetamine has similar rate-dependent effects in schedules that are not based on explicit timing (see Odum 2002, for a recent review). It is also noteworthy that the rate-dependency principle does not only apply to amphetamine; drugs belonging to quite diverse pharmacological classes have been found to exert rate-dependent effects on operant behaviour (see Sanger and Blackman 1976; Dews and Wenger 1977).

Although rate-dependency is a robust finding, subsequent studies indicated that amphetamine may interact more directly with timing processes. Maricq et al. (1981) and Maricq and Church (1983) found that methamphetamine altered performance on the interval bisection task, displacing the psychometric function to the left (i.e. reducing the indifference point, T_{50}), without reducing overall discriminative accuracy. Similarly, in a series of experiments using the fixed-interval peak procedure, Meck (1983; 1986) reported that methamphetamine displaced the response rate function to the left (i.e. reducing the peak time). Interestingly, the reduction of peak time induced by methamphetamine diminished with repeated daily treatment; however, when treatment was subsequently withheld, the peak time increased above its baseline value, before gradually returning again to the baseline.

Meck (1986, 1996; Hinton and Meck 1997) interpreted these results in terms of Scalar Expectancy Theory. He suggested that dopamine release induced by amphetamine-like drugs increases the rate of pulse generation by the hypothetical pacemaker, thereby causing the subject to 'overestimate' the durations of stimuli. Meck further proposed that when the drug is present during repeated training sessions, the animal learns to 'recalibrate' the accumulator, allowing the peak time to resume its optimal location. When the drug is withdrawn, peak time is initially increased due to temporal underestimation, but this is followed by a return to the baseline location as the accumulator is again recalibrated (see Hinton and Meck 1997).

The effects of *d*-amphetamine and methamphetamine on interval timing have been attributed to dopamine release in the striatum (Meck 1986, 1996; Hinton and Meck 1997). However, as well as promoting dopamine release,

amphetamine-like drugs also promote noradrenaline release and, to a lesser extent, 5-HT release, and have some catecholamine uptake blocking action (for review, see Rothman and Baumann 2003). More direct evidence for the involvement of dopaminergic transmission comes from experiments with selective dopamine receptor antagonists. Meck (1986, 1996) reported that several D₂ dopamine receptor antagonists affected peak fixed-interval schedule performance in the opposite direction to amphetamine-like drugs; however, D₁ receptor antagonists were ineffective in this respect. It was therefore proposed that dopaminergic transmission, operating via D₂ dopamine receptors, exerts a facilitatory effect on the hypothetical pacemaker (Meck 1986).

More recently, Meck and his colleagues have developed a revised pacemaker-based model based on the functioning of a cortico-striato-thalamo-cortical circuit (Meck and Benson 2002; Matell and Meck 2000, 2004). The model assumes that neurones in the striatum detect the coincidence of oscillating inputs from the neocortex and thalamus. These oscillating inputs are regarded as constituting the neural basis of the hypothetical pacemaker. Computer simulation studies have shown that a mechanism of this type could generate an output with temporal properties consistent with interval timing behaviour (Matell and Meck 2004), and a recent single unit recording study has identified striatal neurones whose oscillating firing patterns coincide with the duration of a stimulus in a temporal differentiation task (Matell et al. 2003). According to Matell and Meck's (2000, 2004) 'striatal beat frequency model', (see above, 1.4.5), dopaminergic mechanisms contribute to the temporal control of behaviour in two ways: firstly, by adjusting or 'tuning' the pacemaker inputs to the striatum, and secondly by controlling 'attention-

sharing' between temporal processing and other, simultaneous tasks. It is proposed that these two roles of dopamine in timing are mediated by D₂ and D₁ receptors, respectively (Meck and Benson 2002; Buhusi 2003).

This revised pacemaker-based model of timing is the most comprehensive account of the neural substrate of interval timing available at the present time. However, as discussed by Chiang et al. (2000a) and Odum (2002), the principal source of biological evidence supporting all pacemaker models derives from the behavioural effects of amphetamine-like drugs and dopamine receptor antagonists, and this evidence is not entirely consistent. For example, while early studies with the interval bisection task showed that amphetamine-like drugs reduced T_{50} (Maricq et al. 1981; Maricq and Church 1983), other experiments indicated that these drugs produced a general breakdown of temporal discrimination. Thus, an early study by Stubbs and Thomas (1974) showed that *d*-amphetamine dose-dependently reduced pigeons' ability to discriminate two different durations. Similar results were obtained by Rapp and Robbins (1976), and by Santi et al. (1995) using a delayed matching to sample task. More recently, Chiang et al. (2000a) found that *d*-amphetamine produced a dose-dependent increase in the Weber fraction in the interval bisection task (i.e. it reduced discriminative accuracy) without altering T_{50} . Similar findings were reported by Odum et al. (2002) using a free-operant retrospective timing schedule.

The evidence from immediate timing schedules is also ambiguous. Thus, the finding that methamphetamine displaced the peak function to the left (Meck 1983, 1986) has been confirmed by Kraemer et al. (1997) and Buhusi and Meck (2002), but not by Bayley et al. (1998). Chiang et al. (2000a) found

that *d*-amphetamine dose-dependently reduced T_{50} in the free-operant psychophysical procedure, although it also increased the Weber fraction, suggesting that it may also have produced a more general disruption of temporal differentiation.

Chiang et al. (2000a) suggested that amphetamine-like drugs may have different effects on performance in retrospective and immediate timing schedules; in the former case, effects on discriminative accuracy predominate, whereas in the latter case, changes in the locus of the timing function (i.e. peak time or T_{50}) may be more apparent.

Cevik (2003) suggested that other methodological factors may be responsible for the discrepant results. Using an interval bisection task, Cevik (2003) showed that a low dose of *d*-amphetamine (0.5 mg kg^{-1}) shifted the psychometric curve to the left 100 minutes after drug treatment, whereas no such shift was seen at shorter post-injection times (20-100 min).

In conclusion, the evidence reviewed above indicates that performance on timing schedules is sensitive to amphetamine-like drugs and to D_2 receptor antagonists, in accordance with the view that dopaminergic mechanisms contribute to the regulation of timing behaviour. However, at the present time the data are insufficiently consistent to allow a precise definition of dopamine's role in interval timing.

1.5.2. Cholinergic mechanisms and timing

Cholinergic mechanisms are purported to be involved in memory and attentional processes, and disorders of cholinergic function may be associated

with cognitive impairments (see Meck and Church 1987; Sarter and Bruno 1997; Ragozzino 2000). Meck and Church (1987) and Hinton and Meck (1997) proposed a specific role for central cholinergic mechanisms in interval timing: regulation of the transfer of temporal information to and from reference memory.

Unfortunately, there have been relatively few attempts to delineate the effects of manipulating cholinergic function on interval timing performance. Meck and Church (1987) reported that the anticholinesterase physostigmine and the acetylcholine precursor choline ‘sharpened’ the peak of the response rate function in the fixed-interval peak procedure and displaced the function to the left (i.e. reducing the peak time), whereas the muscarinic cholinergic antagonist atropine broadened the peak and displaced it to the right. These effects were interpreted as reflecting reductions and increases, respectively, of remembered durations (Meck and Church 1987).

Meck et al. (1986) reported that systemic treatment with arginine vasopressin, which increased high-affinity choline uptake in the cortex, attenuated the age-related rightward shift of the response rate function, an effect that Meck et al. (1986) interpreted in terms of an improvement of cholinergic transmission-dependent memory function. More recently, Meck and Williams (1997) reported that a dietary choline supplement reversed the effect of choline-deficient diet on peak fixed-interval performance. Meck and Williams (1997) interpreted the effect of the dietary deficiency in terms of an attentional deficit rather than a direct effect on temporal memory.

Odum (2002) examined the effects of atropine and physostigmine on performance on a retrospective timing schedule similar to the interval bisection

task. Physostigmine produced a reduction of T_{50} , consistent with the findings of Meck and Church (1987) with the peak procedure. However, Odum (2002) found that atropine produced an even more marked reduction of T_{50} , the opposite result to that obtained by Meck and Church (1987).

In conclusion, the study of the role of central cholinergic mechanisms in interval timing is insufficiently well developed for any definite conclusions to be drawn. In view of the theoretical importance of cholinergic functions for pacemaker-based timing theories (Hinton and Meck 1997), it would seem that this topic deserves further investigation.

1.5.3. **5-HTergic mechanisms and timing**

There is increasing evidence that the ascending 5-HTergic pathways contribute to the regulation of timing behaviour. However, the precise nature of this contribution remains unclear. Manipulation of 5-HTergic function has been found to have qualitatively different effects on performance in different types of timing schedules (see Al-Ruwaitea et al. 1997; Ho et al. 2002).

Wogar et al. (1992) examined the effect of destruction of the ascending 5-HTergic projection by injection of 5,7-dihydroxytryptamine (5,7-DHT) into the dorsal and median raphe nuclei on performance on an inter-response time schedule (IRT>15-s, or differential-reinforcement-of-low-response-rate [DRL] 15-s). The lesion retarded the acquisition of accurate performance, reduced the mean IRT and increased the Weber fraction. Morrissey et al. (1994) examined the effect of the same lesion on performance on the fixed-interval peak procedure. The peak time was not affected by the lesion, although acquisition

was slowed down and the Weber fraction was increased (see also Al-Ruwaitea et al. 1997). Al-Ruwaitea et al. (1997) speculated that in both these experiments the lesion may have facilitated the rats' propensity to switch between the behavioural states represented by lever pressing and 'other behaviour'. However, this suggestion could not be tested, since both schedules entail the use of a single operandum.

Al-Zahrani et al. (1996) used the free-operant psychophysical procedure to provide direct evidence for an effect of 5-HT depletion on switching between behaviours. The lesion did not prevent the animals from acquiring accurate performance on this schedule, nor did it affect the steady-state values of the indifference point (T_{50}) or the Weber fraction. However, the lesion consistently increased the rate of switching between the two levers.

Chiang et al. (1999) predicted that if switching were restricted to one switch per trial in the 'constrained switching' version of the free-operant psychophysical procedure (Chiang et al. 1998; see above, 1.3.3.5), 5-HT depletion would result in a reduction of T_{50} , due to facilitation of the switch to lever B early in the trial. However, this did not occur (Chiang et al. 1999), suggesting that 5-HT depleted rats are able to learn to withhold switching in immediate timing tasks.

The facilitation of switching induced by 5-HT depletion apparently does not reflect an interaction with timing processes, because Al-Ruwaitea et al. (1999b) found that the lesion markedly enhanced switching between concurrent schedules of reinforcement which did not entail temporal differentiation of behaviour (variable-interval and variable-time schedules).

Several experiments have shown that central 5-HT depletion disrupts

behaviour in prospective timing schedules. Wogar et al. (1993) and Mobini et al. (2000) found that 5-HT depleted rats were less tolerant of delay of reinforcement than sham-lesioned control rats in an adjusting delay schedule (see above, 1.3.4.1.). Similar results were obtained by Al-Ruwaitea et al (1999a) using the 'time-left' procedure (see above, 1.3.4.2).

The experiments reviewed above have shown that total ablation of the 5-HTergic pathways can alter performance on different timing schedules in different ways. However, in no case does 5-HT depletion prevent animals from displaying relatively accurate timing behaviour. More detailed information about the possible role of 5-HTergic neurotransmission in interval timing can be provided by studies of the effects of acute treatment with drugs that interact with specific 5-HT receptors.

Chiang et al. (2000b) examined the effect of acute treatment with the 5-HT_{1A} receptor agonist 8-OH-DPAT on performance on the free-operant psychophysical procedure. The drug produced a dose-dependent leftward shift of the psychometric function, reflected in a reduction of T_{50} . Chiang et al. (2000b) suggested that this effect of 8-OH-DPAT might have been due to stimulation of the somatodendritic autoreceptor population of 5-HT_{1A} receptors. However, Body et al. (2001) found that this was not the case, because the effect of 8-OH-DPAT was not altered by destruction of the 5-HTergic pathways by 5,7-DHT. The effect of 8-OH-DPAT on performance on the free-operant psychophysical procedure could be blocked by the selective 5-HT_{1A} receptor antagonist WAY-100635, indicating that the effect was mediated by 5-HT_{1A} receptors, rather than by 5-HT₇ receptors, for which 8-OH-DPAT also has some affinity (Body et al., 2002b).

Chiang et al. (2000b) found that 8-OH-DPAT also affected performance on the interval bisection task. However, unlike its effect on performance in the free-operant psychophysical procedure, 8-OH-DPAT did not affect T_{50} , but did increase the Weber fraction in this schedule. Body et al. (2002a) obtained similar findings with another retrospective timing schedule, the discrete-trials psychophysical procedure (see above, 1.3.2.3). These findings emphasize the importance of examining the effects of drugs on more than one type of timing schedule. Moreover, according to Chiang et al. (2000a,b), the finding of qualitatively different effects of the same intervention on different timing tasks argues against the possibility that the effects are mediated by an interaction with a ‘unitary’ internal clock (see also Ho et al. 2002).

Body et al. (2003), examined the effect of the mixed 5-HT_{2A/2C} receptor agonist, 2,5-dimethoxy-4-iodoamphetamine (DOI), and antagonist, ketanserin, on temporal differentiation in the free-operant psychophysical procedure. Body et al. (2003) speculated that 5-HT_{1A} receptors might be subordinate to a prepotent, and functionally opposite, population of 5-HT₂ receptors. This speculation, however, was not confirmed by their finding that DOI had a qualitatively similar effect on temporal differentiation to that produced by 8-OH-DPAT (i.e. DOI dose-dependently reduced T_{50}). The reduction of T_{50} by DOI was reversed by ketanserin; since ketanserin has an approximately 80-fold higher affinity for 5-HT_{2A} receptors than for 5-HT_{2C} receptors (Barnes and Sharp 1999; Hoyer et al. 2002), this finding implicates 5-HT_{2A} receptors in DOI’s effect. It is not known whether 5-HT_{2A} receptor stimulation affects

temporal discrimination performance in the same way that it alters temporal differentiation.

The results discussed above indicate that temporal differentiation in the free-operant psychophysical procedure is sensitive to stimulation of both 5-HT_{1A} and 5-HT_{2A} receptors; in both cases, the receptor population appears to be postsynaptic. Body et al. (2004) investigated whether either or both these receptor populations can be stimulated by endogenously released 5-HT. These authors found that the 5-HT releasing agent fenfluramine, like 8-OH-DPAT and DOI, displaced the psychometric curve to the left, reducing T_{50} . This effect of fenfluramine was absent in rats whose 5-HTergic pathways had been ablated by intra-raphe injection of 5,7-DHT, confirming that the effect depended on the existence of intact 5-HTergic neurones, and suggesting that it was mediated by the release of endogenous 5-HT. The effects of both 8-OH-DPAT and DOI were unaffected by the lesion, confirming that these agonists' effects were mediated by post-synaptic receptor populations. Interestingly, fenfluramine's effect could be antagonized by ketanserin but not by WAY-100635, suggesting that endogenous 5-HT influenced temporal differentiation principally via an interaction with 5-HT_{2A} rather than 5-HT_{1A} receptors.

In conclusion, the experiments reviewed in this section indicate that, while the integrity of the 5-HTergic pathways is not essential for the accurate performance of timing tasks, acute stimulation of at least two subtypes of 5-HT receptor, 5-HT_{1A} and 5-HT_{2A} receptors, can have marked effects on timing behaviour. In the case of 5-HT_{1A} receptor stimulation, there is an interesting disjunction between the effects on temporal differentiation and temporal discrimination: in the former case, the psychometric function is displaced to the

left (i.e. T_{50} is reduced), whereas in the latter case the main effect is a flattening of the function (i.e. the Weber fraction is increased). The implications of these findings for the neural mechanisms underlying interval timing behaviour have not yet been fully worked out. However, Ho et al. (2002) have argued that the qualitatively different effects of the same drug treatment on different timing tasks are difficult to reconcile with the notion of a unitary internal clock, such as that proposed by SET and BET, which is assumed to subserve timing behaviour in all types of timing schedule.

CHAPTER 2

Experiment 1:

EFFECTS OF QUIPAZINE AND *m*-CHLOROPHENYLBIGUANIDE (*m*-CPBG) ON TEMPORAL DISCRIMINATION

2.1. INTRODUCTION

Two major classes of timing schedule are *immediate* and *retrospective* timing schedules (Killeen and Fetterman 1988; Killeen et al. 1997; see above, section 1.3). Immediate timing schedules require the organism to regulate its own behaviour in time (*temporal differentiation*), whereas retrospective timing tasks entail discrimination of the durations of exteroceptive stimuli (*temporal discrimination*). Examples of immediate timing schedule are the free-operant psychophysical procedure and fixed-interval peak procedure (see section 1.3.3). An example of a retrospective timing schedule is the discrete-trials psychophysical procedure (Body et al. 2002a; see section 1.2.3.3). In this schedule, a light is presented for a variable time, $t < 50$ s, following which two levers are offered; a response on A is reinforced if $t < 25$ s, whereas a response on B is reinforced if $t > 25$ s. Temporal discrimination is measured quantitatively from a sigmoid psychometric curve derived from the proportional choice of B as a function of t . In both types of schedule, the psychometric curve conforms to a two-parameter logistic function from which indices of timing are derived which reflect its central tendency (the indifference point, T_{50} , or ‘point of subjective equality’, PSE: see Killeen et al. 1997) and its variability (the Weber fraction) (see Killeen et al. 1997; Ho et al. 2002).

Despite the superficial similarity of the timing indices derived from immediate and retrospective timing tasks, they differ in their sensitivities to pharmacological challenge (see section 1.5.3.). For example, the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) has

been found to reduce T_{50} without altering the Weber fraction in an immediate timing schedule, but to increase the Weber fraction without altering T_{50} in retrospective timing schedules (Chiang et al. 2000, Body et al. 2001, 2002a, 2002b, 2004). Both these effects are apparently mediated by postsynaptic receptor populations, since they are impervious to destruction of the ascending 5-HTergic pathways (Body et al. 2001, 2002a, 2002b, 2004).

It is not known whether 5-HT₃ receptor stimulation has any systematic effect on temporal discrimination. The present experiment examined the effects of *m*-chlorophenylbiguanide (*m*-CPBG) and quipazine on performance in the discrete-trials psychophysical procedure. *m*-CPBG is a selective 5-HT₃ receptor agonist (Kilpatrick et al. 1990); quipazine has nanomolar affinity for 5-HT₃ receptors and micromolar affinity for 5-HT_{2A} receptors (Hoyer 1988; Glennon et al. 1989; Sharif et al. 1991). The sensitivity of quipazine's effects to antagonism by the 5-HT₃ receptor antagonist MDL-72222 (Fozard 1984) and the 5-HT_{2A} receptor antagonist ketanserin (Barnes and Sharp 1999; Hoyer et al. 2002) was also examined.

2.2. METHODS

2.2.1. Subjects

Twenty-four female Wistar rats, aged approximately 4 months and weighing 250-290 g at the start of the experiment, were housed individually under a constant cycle of 12 h light and 12 h darkness (lights on 07.00-19.00 hours), and were maintained at 80% of their initial free-feeding body weights by

providing a limited amount of standard rodent diet after each experimental session. Tap water was freely available in the home cages.

2.2.2. **Apparatus**

The rats were trained in operant conditioning chambers (Campden Instrument Limited, Sileby, UK) of internal dimensions 20 cm × 23 cm × 22.5 cm. One wall of the chamber contained a recess into which a motor-operated dipper could deliver 50 µl of a liquid reinforcer. Apertures were situated 5 cm above and 2.5 cm on either side of the recess; a motor-driven retractable lever could be inserted into the chamber through each aperture. Each lever could be depressed by a force of approximately 0.2 N. The chamber was enclosed in a sound-attenuating chest; masking noise was provided by a rotary fan. Twelve chambers were used; each rat was always tested in the same chamber. An Acorn microcomputer programmed in Arachnid BASIC (CeNeS Ltd, Cambridge, UK), located in an adjoining room controlled the schedules and recorded the behavioural data.

2.2.3. **Behavioural training**

At the start of the experiment, the food-deprivation regimen was started and the rats were gradually reduced to 80% of their free-feeding body weights. They were then trained to press the levers, and were exposed to a discrete-trials continuous reinforcement schedule, in which the two levers were presented in random sequence, for three sessions. Thereafter, the rats underwent 50-minute

training sessions under the discrete-trials psychophysical procedure, 7 days a week, at the same time each day during the light phase of the daily cycle (between 8.00 and 13.00 hours). The reinforcer, a 0.6 M solution of sucrose in distilled water, was prepared daily before each session.

The discrete-trials psychophysical procedure was identical to that described by Body et al. (2002). Each session consisted of fifty trials, successive trials being initiated at 60-s intervals. Each trial started with the illumination of a lamp above the central reinforcer recess. After a predetermined interval had elapsed (see below), the levers were inserted into the chamber. A single response on either lever resulted in withdrawal of both levers and extinguishing of the light; the chamber remained in darkness until the start of the next trial. Lever insertion took place once in each trial, at one of the following “entry points” following the start of the trial: 2.5, 7.5, 12.5, 17.5, 22.5, 27.5, 32.5, 37.5, 42.5, 47.5 s. If lever insertion took place at any of the first five entry points (i.e., less than 25 s after trial onset), a response on lever A resulted in reinforcer delivery, whereas a response on lever B did not; conversely, if lever insertion took place at any of the last five entry points (i.e., more than 25 s after trial onset), a response on lever B resulted in reinforcer delivery, whereas a response on lever A did not. If no response occurred within 5 s of lever insertion, the levers were withdrawn and the light was extinguished (this seldom occurred after the first few sessions of training). The positions of levers A and B (left versus right) were counterbalanced across subjects. In each session, there were 40 trials in which both levers were presented (four trials with each entry point, in pseudo-random sequence). The remaining trials were forced-choice trials in which only one lever was presented (lever A in five

trials and lever B in the other five), the entry points occurring in a pseudo-random sequence.

2.2.4. Drug treatment

The drug treatment regimen started after 90 sessions of preliminary training under the discrete-trials psychophysical procedure. Injections of drugs were given on Tuesdays and Fridays, and injections of the vehicle alone (0.9% sodium chloride solution) on Mondays and Thursdays; no injections were given on Wednesdays, Saturdays or Sundays. Each dose of each drug was administered five times, in order to accrue a sufficient number of trials for function fitting (see below). The order of treatments within each series was balanced within and between animals according to a Latin square. Subcutaneous injections were given using a 26-gauge needle at a volume of 1.0 ml kg⁻¹; intraperitoneal injections were given using a 25-gauge needle at a volume of 2.5 ml kg⁻¹. The doses of the drugs tested were as follows (doses were selected on the basis of previous behavioural studies with rats: see Discussion for references).

Agonists: quipazine dimaleate, 0.5, 1, 2 mg kg⁻¹; *m*-chlorophenylbiguanide hydrochloride (*m*-CPBG), 2.5, 5, 10 mg kg⁻¹. Both drugs were dissolved in 0.9% sodium chloride solution; quipazine was administered subcutaneously 15 min before the start of the experimental session, and *m*-CPBG intraperitoneally 30 min before the start of the session.

Antagonists: topanyl 3,5-dichlorobenzoate (MDL-72222), 0.25, 0.5, 1 mg kg⁻¹ was dissolved in glacial acetic acid, buffered to pH 5.5, and diluted

with 0.9% sodium chloride to give the desired concentration; it was injected intraperitoneally 30 min before the start of the session. Ketanserin tartrate, 0.5, 1, 2 mg kg⁻¹ was dissolved in 0.9% sodium chloride; it was injected subcutaneously 15 min before the start of the session.

Agonist/antagonist interaction: Quipazine 2 mg kg⁻¹ was administered alone and in combination with either MDL-72222 1 mg kg⁻¹ or ketanserin 2 mg kg⁻¹.

2.2.5. Data analysis

Separate analyses were carried out on the effects of quipazine, *m*-CPBG, MDL-72222 and ketanserin, and the interaction between quipazine, MDL-72222 and ketanserin.

For each treatment condition, the percentages of responses emitted on lever B (%B) at each time-point were analysed by two-factor analyses of variance (treatment × time) with repeated measures on both factors. In the event of a significant main effect of treatment or a significant treatment × time interaction, analyses of the simple main effect at each time-point were carried out, followed by comparisons between each active treatment with the vehicle-alone condition using Dunnett's test.

For the quantitative analysis of the psychometric functions (%B plotted against stimulus duration, *t*), two-parameter logistic functions were fitted to the data obtained under each treatment condition: $\%B = 100/(1+[t/T_{50}]^\varepsilon)$, where T_{50} (indifference point) is the stimulus duration corresponding to %B = 50%, and ε is the slope of the function (Al-Zahrani et al. 1996). The curve-fitting

procedure yields estimates (\pm SE est.) of the values of T_{50} and ϵ , from which the Weber fraction was determined as follows. The limen was defined as half the difference between T_{75} and T_{25} (T_{75} and T_{25} being the values of t corresponding to $\%B=75\%$ and $\%B=25\%$), and the Weber fraction was calculated as the ratio of the limen to T_{50} . Goodness of fit of the logistic functions was expressed as the index of determination, p^2 . The values of T_{50} , ϵ , and the Weber fraction were analysed by one-factor analyses of variance (treatments) with repeated measures. In the case of a significant effect of treatment, comparisons were made between each active treatment and the control (vehicle alone) condition using Dunnett's test (significance criterion, $P<0.05$). In the case of data from the quipazine-ketanserin/MDL-72222 interaction, multiple comparisons were made between treatment with quipazine alone and the combined quipazine + antagonist treatments, using Neuman-Keul's test (significance criterion, $P<0.05$).

2.3. RESULTS

Under each treatment condition, the proportion of responding allocated to lever B ($\%B$) increased progressively as a function of stimulus duration, t . Under the vehicle-alone condition and all active treatment conditions, the number of 'missed' trials (i.e. trials in which no response was emitted on either lever A or lever B) was $<0.5\%$.

2.3.1. Effects of the agonists

Quipazine. The effect of quipazine on proportional choice of lever B

(%B) is shown in Fig. 2.1.A. Analysis of variance of these data revealed significant main effects of treatment [$F(3,69) = 12.0$, $P < 0.001$] and time [$F(9,207) = 556.6$, $P < 0.001$], and a significant treatment \times time interaction [$F(27,621) = 8.2$, $P < 0.001$]. Analysis of the simple main effects revealed significant treatment effects 7.5 s after trial onset, and at all time points > 25 s after trial onset. Multiple comparisons with the vehicle-alone condition showed that quipazine 2 mg kg^{-1} produced a significant increase in %B 7.5 s after trial onset and significant decreases in %B at all time points > 25 s after trial onset. Quipazine 1 mg kg^{-1} produced decreases in %B 27.5, 32.5, 37.5 and 47.5 s after trial onset.

Logistic functions were fitted to the data from each rat under each treatment condition; the group mean values of parameters of these functions (\pm SEM) are shown in Table 2.1. Quipazine flattened the psychometric function and displaced it rightwards; these effects are reflected in the parameter values. There was a dose-dependent increase in the value of T_{50} ; analysis of variance showed a significant effect of treatment [$F(3,69) = 10.9$, $P < 0.001$], and multiple comparisons with the vehicle-alone condition indicated that quipazine 1 and 2 mg kg^{-1} significantly increased T_{50} . There was also a significant effect on the slope of the function, ϵ [$F(3,69) = 16.5$, $P < 0.001$], the effects of 1 and 2 mg kg^{-1} being statistically significant. The Weber fraction was increased by quipazine [$F(3,69) = 4.1$, $P < 0.01$], the effect of 2 mg kg^{-1} being significant.

m-CPBG. The %B data are shown in Fig. 2.1.B. Analysis of variance of these data revealed a significant main effect of time [$F(9,207) = 1085.3$, $P < 0.001$], but no significant main effect of treatment [$F(3,69) = 1.1$, $P > 0.1$] and no significant treatment \times time interaction [$F(27,621) = 1.5$, $P > 0.1$].

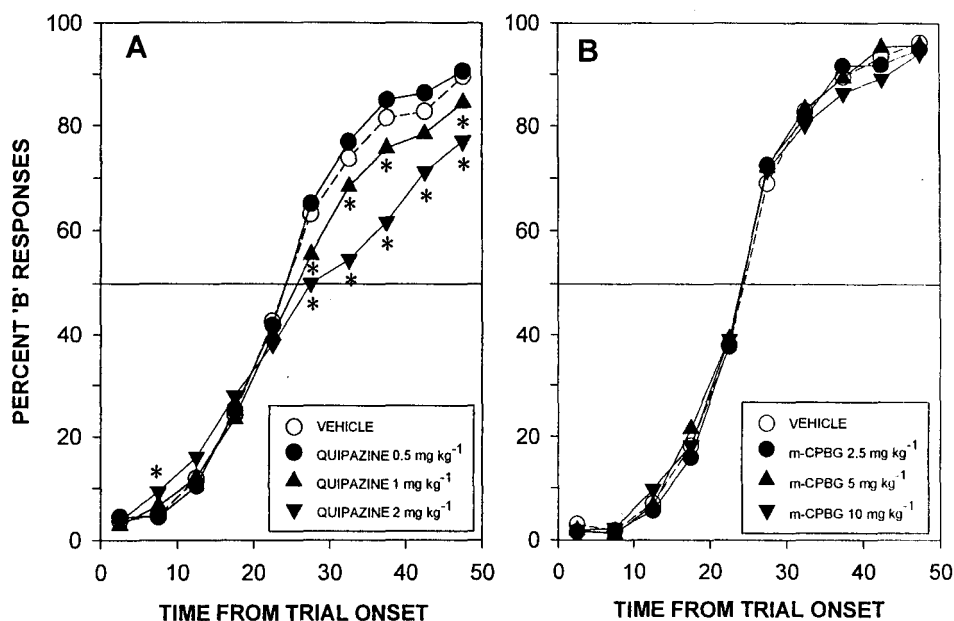


Figure 2.1. **A.** Effect of quipazine on relationship between proportional choice of lever B (%B) and stimulus duration (t , seconds) in the discrete-trials psychophysical procedure. Points indicate group mean data under each treatment condition (see inset). Significance of difference from vehicle-alone treatment: * $P < 0.05$. **B** Effect of *m*-CPBG on relationship between proportional choice of lever B and stimulus duration; conventions as in A.

Table 2.1. Effects of the agonists on measures of performance on the discrete-trials psychophysical procedure: group mean values (\pm SEM)

Treatment	Parameters of logistic psychometric function			
	T_{50} , s	slope, ϵ	p^2	Weber fraction
Vehicle	24.0 \pm 0.7	-3.8 \pm 0.2	0.94 \pm 0.04	0.31 \pm 0.02
quipazine 0.5 mg kg ⁻¹	24.4 \pm 0.7	-3.6 \pm 0.3	0.95 \pm 0.01	0.37 \pm 0.04
quipazine 1 mg kg ⁻¹	26.1 \pm 0.7 *	-3.1 \pm 0.2 *	0.93 \pm 0.02	0.43 \pm 0.02 *
quipazine 2 mg kg ⁻¹	30.2 \pm 1.5 *	-2.3 \pm 0.2 *	0.89 \pm 0.02	0.84 \pm 0.25 *
Vehicle	24.1 \pm 0.4	-5.7 \pm 0.4	0.99 \pm 0.00	0.21 \pm 0.01
<i>m</i> -CPBG 2.5 mg kg ⁻¹	24.4 \pm 0.5	-6.8 \pm 0.7	0.97 \pm 0.00	0.20 \pm 0.02
<i>m</i> -CPBG 5 mg kg ⁻¹	23.6 \pm 0.5	-5.5 \pm 0.4	0.98 \pm 0.00	0.22 \pm 0.01
<i>m</i> -CPBG 10 mg kg ⁻¹	24.0 \pm 0.6	-5.4 \pm 0.5	0.97 \pm 0.01	0.24 \pm 0.02

Significance of difference from vehicle condition, * $P < 0.05$

The parameters of the logistic functions are shown in Table 2.1. *m*-CPBG had no significant effect on the values of T_{50} [$F(3,69) = 1.5$, $P>0.05$], ε [$F(3,69) = 2.4$, $P>0.05$] or the Weber fraction [$F(3,69) = 2.4$, $P>0.05$].

2.3.2. Effects of the antagonists

MDL-72222. The %B data are shown in Fig. 2.2.A. Analysis of variance of these data revealed a significant main effect of time [$F(9,207) = 967.0$, $P<0.001$], but no significant main effect of treatment [$F(3,69) = 1.7$, $P>0.1$] and no significant treatment \times time interaction [$F<1$].

The parameters of the logistic functions are shown in Table 2.2. *MDL-72222* had no significant effect on the values of T_{50} [$F(3,69) = 2.3$, $P>0.05$] or ε [$F(3,69) = 2.2$, $P>0.05$]. There was a significant treatment effect in the case of the Weber fraction [$F(3,69) = 4.3$, $P<0.01$]; however this did not appear to be dose-related, and none of the individual doses of *m*-CPBG differed significantly from the vehicle-alone condition.

Ketanserin. The %B data are shown in Fig. 2.2.A. Analysis of variance of these data revealed a significant main effect of time [$F(9,207) = 1050.3$, $P<0.001$], but no significant main effect of treatment [$F(3,69) = 1.6$, $P>0.1$] and no significant treatment \times time interaction [$F<1$].

The parameters of the logistic functions are shown in Table 2.2. *Ketanserin* had no significant effect on the values of T_{50} [$F(3,69) = 2.1$, $P>0.05$] or ε [$F(3,69) = 1.5$, $P>0.05$] or the Weber fraction [$F(3,69) = 0.6$, $P>0.05$].

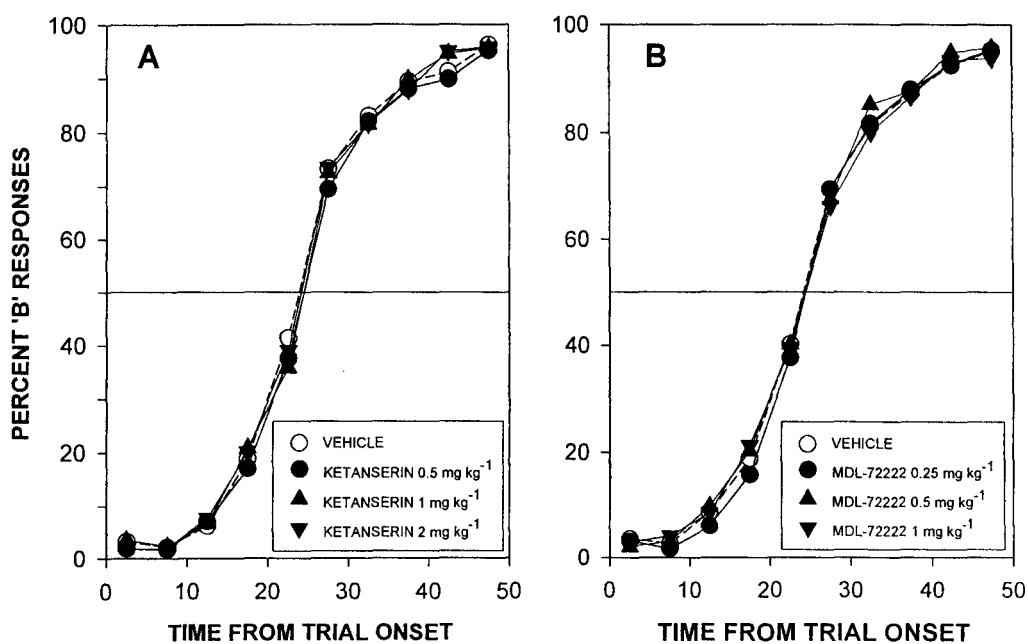


Figure 2.2. A. Effect of ketanserin on relationship between proportional choice of lever B and stimulus duration. Conventions as in Fig. 2.1. B. Effect of MDL-72222 on relationship between proportional choice of lever B and stimulus duration

Table 2.2. Effects of the antagonists on measures of performance in the discrete-trials psychophysical procedure: group mean values (\pm SEM)

Treatment	Parameters of logistic psychometric function			
	T_{50}, s	slope, ϵ	p^2	Weber fraction
Vehicle	23.9 \pm 0.4	-5.0 \pm 0.3	0.98 \pm 0.00	0.24 \pm 0.02
MDL-72222 0.25 mg kg ⁻¹	24.7 \pm 0.5	-5.8 \pm 0.4	0.97 \pm 0.01	0.21 \pm 0.02
MDL-72222 0.5 mg kg ⁻¹	23.7 \pm 0.4	-6.2 \pm 0.8	0.97 \pm 0.00	0.23 \pm 0.02
MDL-72222 1 mg kg ⁻¹	23.8 \pm 0.6	-4.7 \pm 0.4	0.97 \pm 0.01	0.27 \pm 0.02
Vehicle	23.6 \pm 0.5	-5.4 \pm 0.4	0.99 \pm 0.00	0.22 \pm 0.01
ketanserin 0.5 mg kg ⁻¹	24.5 \pm 0.5	-5.5 \pm 0.4	0.97 \pm 0.00	0.22 \pm 0.02
ketanserin 1 mg kg ⁻¹	24.1 \pm 0.5	-6.3 \pm 0.7	0.97 \pm 0.00	0.20 \pm 0.02
ketanserin 2 mg kg ⁻¹	23.6 \pm 0.6	-5.9 \pm 0.4	0.97 \pm 0.00	0.21 \pm 0.01

2.3.3. Agonist-antagonist interaction

The %B data are shown in Fig. 2.3. Analysis of variance of these data revealed significant main effects of treatment [$F(3,69) = 16.9, P<0.001$] and time [$F(9,207) = 585.5, P<0.001$], and a significant treatment \times time interaction [$F(27,621) = 16.8, P<0.001$]. Analysis of the simple main effects revealed significant treatment effects 7.5 and 12.5 s after trial onset, and at all time points >25 s after trial onset. Multiple comparisons with the vehicle-alone condition showed that quipazine and quipazine + MDL-72222 produced significant increases in %B 7.5 and 12.5 s after trial onset and significant decreases in %B at all time points >25 s after trial onset. In no case did quipazine + ketanserin differ significantly from vehicle-alone treatment, and there were no significant differences between the quipazine and quipazine + MDL-72222 treatment conditions.

The parameters of the logistic functions are shown in Table 2.3. There was a significant effect of treatment on T_{50} [$F(3,69) = 7.9, P<0.001$]. Multiple comparisons with the vehicle condition (Dunnett's test) showed that T_{50} was significantly increased by quipazine and by quipazine + MDL-72222, but not significantly changed by quipazine + ketanserin. Multiple comparisons with the quipazine condition (Newman-Keul's test) showed that the increase in T_{50} produced by quipazine was significantly reversed by combined treatment with ketanserin but not by combined treatment with MDL-72222.

There was a significant effect of treatment on ϵ [$F(3,69) = 42.7, P<0.001$]. Multiple comparisons with the vehicle alone condition showed that

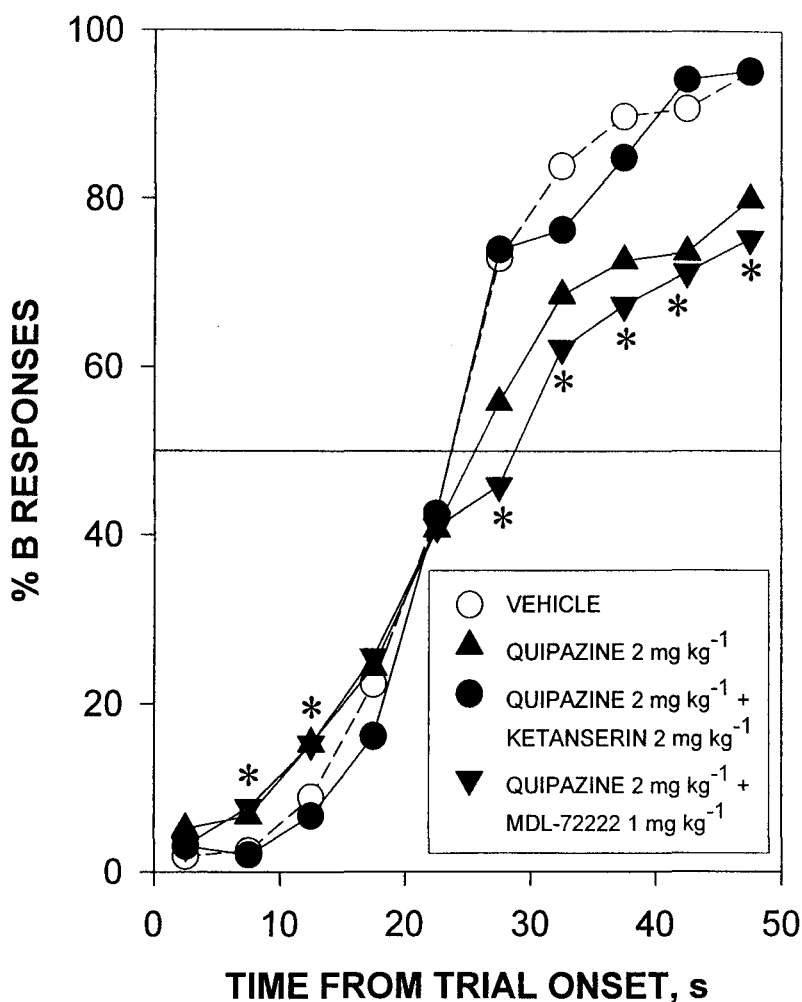


Figure 2.3. Interaction between quipazine (QUIP), MDL-72222 (MDL) and ketanserin (KET) on relationship between proportional choice of lever B and stimulus duration in the discrete-trials psychophysical procedure. Conventions as in Fig.2.1. The psychometric function was flattened and displaced to the right by quipazine; this effect was reversed by co-administration of ketanserin, but not by co-administration of MDL-72222. Significance of difference from vehicle-alone treatment: * $P < 0.05$; note that asterisks refer to both the quipazine and quipazine + MDL-72222 treatments.

Table 2.3. Interaction between quipazine and the antagonists on measures of performance in the discrete-trials psychophysical procedure: group mean values (\pm SEM)

Treatment	Parameters of logistic psychometric function			
	T_{50} , s	slope, ϵ	P^2	Weber fraction
Vehicle	23.4 \pm 0.4	-5.1 \pm 0.3	0.94 \pm 0.04	0.21 \pm 0.01
quipazine 2 mg kg ⁻¹	25.9 \pm 0.8 *	-2.8 \pm 0.2 *	0.90 \pm 0.02	0.60 \pm 0.13 *
quipazine 2 mg kg ⁻¹ + MDL-72222 1 mg kg ⁻¹	28.1 \pm 1.5 *	-2.6 \pm 0.3 *	0.97 \pm 0.01	0.70 \pm 0.18 *
quipazine 2 mg kg ⁻¹ + ketanserin 2 mg kg ⁻¹	23.9 \pm 0.6 #	-5.3 \pm 0.3 #	0.89 \pm 0.03	0.22 \pm 0.01 #

Significance of difference from vehicle condition, * $P < 0.05$; significance of difference from quipazine 2 mg kg⁻¹ condition, # $P < 0.05$

the value of ϵ was increased both by quipazine and by quipazine + MDL-72222, but not by quipazine + ketanserin. Multiple comparisons with the quipazine condition showed that quipazine's effect on the parameter was reversed by ketanserin but not by MDL-72222.

There was a significant effect of treatment on the Weber fraction [$F(3,69) = 7.1, P < 0.001$]. Multiple comparisons with the vehicle alone condition showed that quipazine and quipazine + MDL-72222 increased the Weber fraction. Multiple comparisons with the quipazine condition showed that the increase in the Weber fraction produced by quipazine was significantly reversed by ketanserin but not by MDL-72222.

2.4. DISCUSSION

Temporal discrimination in the discrete-trials psychophysical procedure seen in these experiments was similar to that reported previously: proportional choice of lever B increased as a sigmoid function of stimulus duration, this being well described by a two-parameter logistic equation (Body et al. 2002).

Quipazine (0.5-2.0 mg kg⁻¹) produced a dose-dependent disruption of temporal discrimination, which was most readily apparent in the case of longer stimulus durations. This resulted in rightward displacement and flattening of the fitted psychometric function, reflected in a significant increase in the value of T_{50} , combined with significant increases in ϵ and the Weber fraction. The increase in the Weber fraction produced by quipazine indicates an impairment of the precision with which the rats discriminated the durations of the light stimulus (see Killeen et al. 1997). The origin of the increase in T_{50} induced by quipazine remains unclear. Acute changes in T_{50} are often ascribed to changes

in the period of the hypothetical internal pacemaker, the putative substrate of interval timing behaviour (see Gibbon et al. 1997a; see below). However, other explanations for quipazine's effect on T_{50} may be possible. For example, the increases in T_{50} and the Weber fraction may both be explained in terms of a general breakdown of stimulus control induced by the drug, if it is assumed that stimulus control is weaker, and therefore more vulnerable to disruption, during the longer stimulus durations. Further experiments will be needed to address this issue; one obvious question to ask is whether quipazine's effects on discrimination performance are specific to the temporal dimension, or whether discriminative control exerted by other stimulus dimensions may be equally sensitive to this drug.

The effect of quipazine on temporal discrimination performance was not shared by the selective 5-HT₃ receptor agonist *m*-CPBG (Kilpatrick et al. 1990). It is unlikely that the lack of effect of *m*-CPBG reflects the use of an inadequate dose of this drug, since the dose range used in this experiment has been found to be active in other behavioural paradigms (Higgins et al. 1993). The lack of effect of *m*-CPBG stands in contrast to the robust effect of quipazine. Quipazine has a very high affinity for 5-HT₃ receptors ($K_d = 2$ nM: Glennon et al. 1989; Sharif et al. 1991), and somewhat lower affinity (micromolar range) for several other subtypes of 5-HT receptor, including 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors (Hoyer 1988). Quipazine's effect on temporal discrimination was not altered by co-administration of MDL-72222, but was completely abolished by co-administration of ketanserin. MDL-72222 is a selective 5-HT₃ receptor antagonist (Fozard 1984); the doses used in this experiment have been shown to be behaviourally active in a variety of tests that

are thought to reflect 5-HT₃ receptor function (Costall and Naylor 1992; Higgins et al. 1992; Mazzola-Pomietto et al. 1995). Taken together, these findings suggest that central 5-HT₃ receptor stimulation does not influence temporal discrimination. Furthermore, the complete reversal of quipazine's effect by ketanserin strongly implicates 5-HT₂ receptors in this effect. A contribution of 5-HT_{1B} receptors is rendered unlikely by the fact that ketanserin has a rather low affinity for 5-HT₁ receptors compared to its very high affinity for 5-HT₂ receptors (Leysen et al. 1981; Zgombick et al. 1995). No firm statement can be made about the nature of ketanserin's interaction with quipazine in the present experiments, since only a single dose of the agonist and antagonist were tested. However, there is good evidence that ketanserin is a competitive antagonist at 5-HT₂ receptors both in the periphery (van Nueten et al. 1981) and in the central nervous system (Branchek et al. 1990). Since ketanserin has an 80-100 times higher affinity for the 5-HT_{2A} receptor than for the 5-HT_{2C} receptor (Baxter et al. 1995; Barnes and Sharp 1999), it is likely that the effect of quipazine is mediated by 5-HT_{2A} receptors (see Body et al. 2003). Confirmation of this suggestion will require further experiments using more selective 5-HT_{2A} and 5-HT_{2C} receptor antagonists.

In a previous experiment, Body et al. (2002) found that the 5-HT_{1A} receptor agonist 8-OH-DPAT also impaired temporal discrimination in the discrete-trials psychophysical procedure (Body et al. 2002a). This effect of 8-OH-DPAT was evidently mediated by a postsynaptic receptor population, since the effect survived destruction of the ascending 5-HTergic pathways by intraraphe injection of the selective neurotoxin 5,7-dihydroxytryptamine. The present findings suggest that 5-HT_{2A} and 5-HT_{1A} receptors mediate

qualitatively similar effects on temporal discrimination. Whether or not the two receptor subtypes reside on the same population of neurones is a question for future research.

The present results implicate 5-HT_{2A} receptors in quipazine's effect on temporal discrimination. 5-HT_{2A} receptors are widely distributed in the brain (see Hoyer et al. 2002), including areas, such as the basal ganglia, that are thought to play a significant role in the control of timing behaviour (Gibbon et al. 1997a; Matell and Meck 2000, 2004). Future experiments employing intracerebral injection of agonists may help to elucidate the location of the receptor population underlying quipazine's effects on temporal discrimination.

The increase in T_{50} produced by quipazine was somewhat greater in the first phase of the experiment (from 24.0 to 30.2) than in the final phase (from 23.4 to 25.9). The reason for this difference is not clear. However, the fact that significant increases were seen in both phases suggests that it is a qualitatively reliable effect. The rightward displacement of the psychometric function seen in this experiment stands in contrast to the leftward displacement of the curve (reduction of T_{50}) produced by the 5-HT_{2A/2C} receptor agonist DOI in a previous experiment using the free-operant psychophysical procedure (Body et al. 2003). Assuming that quipazine was acting via 5-HT₂ receptors in this experiment, as argued above, this is a surprising observation that may have implications not only for the role of 5-HT in interval timing behaviour but also for theoretical models of interval timing. Most current models of interval timing assume, either tacitly or explicitly, that a single central timekeeper or internal clock is engaged in all forms of timing performance, including both temporal discrimination and temporal differentiation (see Zeiler 1998;

Grondin 2001; Ho et al. 2002). The notion of a unitary internal clock has recently been questioned on psychophysical grounds, in the light of evidence for systematic quantitative differences in the precision of timing revealed by different types of timing task (Grondin 2001), and some authors have doubted the necessity for positing any kind of clock-like mechanism underlying animals' timing performance (e.g. Zeiler 1998). Pharmacological evidence may also be pertinent to this debate (Ho et al. 2002). The finding of systematic qualitative differences between the effects of some psychoactive drugs on temporal discrimination and temporal differentiation suggests either that these drugs interact with distinct timekeepers that may underlie the two types of interval timing behaviour, or that the drugs influence some "non-timing" processes that are invoked to differing degrees by the different types of timing task. For example, one obvious difference between the free-operant and discrete-trials psychophysical procedures is the occurrence of repetitive responding in the former schedule but not in the latter. Other differences include the possibility of switching from one operandum to the other in the free-operant, but not the discrete-trials schedule, and possible differences between the rates or probabilities of reinforcement provided by the two types of schedules (see Ho et al. 2002). Whatever factors are ultimately found to be responsible for the diverse effects of drugs on temporal discrimination and temporal differentiation, it is apparent that general conclusions about the neurobiological substrate of interval timing cannot be derived from examination of the effects of drugs on any one timing task (see Ho et al. 2002).

CHAPTER 3

Experiment 2:

EFFECTS OF QUIPAZINE AND *m*-CHLOROPHENYLBIGUANIDE (*m*-CPBG) ON TEMPORAL DIFFERENTIATION

3.1. INTRODUCTION

Experiment 1, described in Chapter 2, examined the effects of quipazine and *m*-CPBG on temporal discrimination in the discrete-trials psychophysical procedure (Body et al. 2002a). The experiment described in this chapter examined the effects of the same drugs on *temporal differentiation* in the free-operant psychophysical procedure (Stubbs 1976; Chiang et al. 1998).

Temporal differentiation, one of the principal classes of interval timing behaviour (see section 1.3), entails the temporal regulation of behaviour during an ongoing time interval. It is revealed by *immediate timing schedules* (Killeen and Fetterman 1988; Killeen et al. 1997), such as the free-operant psychophysical procedure (Stubbs 1976). In this schedule the subject is presented with two levers, one of which (A) provides reinforcement during the first half of a trial, while the other (B) provides reinforcement during the second half of a trial. Typical performance on this schedule consists of a declining response rate on lever A and a concomitantly increasing response rate on lever B as the trial progresses. Temporal differentiation is measured quantitatively from the sigmoid psychometric curve, which is derived from the proportion of responding directed towards lever B (%B) over the course of the trial. This curve is well described by the same two parameter logistic function that is used to describe temporal discrimination performance (Killeen et al. 1997; Ho et al 2002).

As reviewed in section 1.5, temporal differentiation in the free-operant psychophysical procedure is sensitive to 5-HT_{1A} and 5-HT_{2A} receptor stimulation. The 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) disrupts performance on this schedule, displacing the psychometric curve to the left and reducing T_{50} , an effect that can be blocked by the selective 5-HT_{1A}

receptor antagonist *N*-[2-(4-[2-methoxyphenyl]-1-piperazinyl)ethyl]-*N*-2-pyridinyl-cyclohexanecarboxamide (WAY-100635) (Chiang et al. 2000b; Body et al. 2002a, 2004).

Recently Body et al. (2003, 2004) found that the 5-HT_{2A/2C} receptor agonist, 2,5-dimethoxy-4-iodoamphetamine (DOI), and the 5-HT releasing agent, fenfluramine, also reduced T_{50} , effects that could be blocked by the selective 5-HT_{2A} receptor antagonist ketanserin but not by WAY-100635 (Body et al. 2003, 2004). These findings indicate that stimulation of either 5-HT_{1A} or 5-HT_{2A} receptors can influence temporal differentiation.

The aim of the present experiments was to examine whether temporal differentiation is sensitive to stimulation of 5-HT₃ receptors. The effects of two 5-HT₃ receptor agonists were examined on temporal differentiation performance; it was also examined whether the effects of these agonists could be reversed by a 5-HT₃ and/or a 5-HT_{2A} receptor antagonist. The agonists and antagonists used in this experiment were the same as those used in Experiment 1.

3.2. METHOD

3.2.1. Subjects

Twenty-four female Wistar rats aged approximately 4 months and weighing 250-290 g at the start of the experiment, were housed under the same conditions as those used in Experiment 1.

3.2.2. Apparatus

The rats were trained in standard operant conditioning chambers (Campden

Instruments Limited, Sileby, UK) (see Section 2.2.2, for description).

3.2.3. **Behavioural training**

Two weeks before starting the experiment, the food deprivation regimen was introduced and the rats were gradually reduced to 80% of their free-feeding body weights. Then they were trained to press the lever for the sucrose reinforcer, and were exposed a discrete-trials continuous reinforcement schedule, in which the two levers were presented in random sequence, for three sessions. The rats then underwent 50-minute training sessions in the free-operant psychophysical procedure, 7 days a week, at the same time each day during the light phase of the daily cycle (between 0800 and 1300 hours), for the remainder of the experiment. The reinforcer, a 0.6 M solution of sucrose in distilled water, was prepared daily before each session.

The free-operant psychophysical procedure used was identical to that used by Chiang et al. (1998, 2000a, 2000b). Each session consisted of fifty 50-s trials, successive trials being separated by 10-s intertrial intervals. In 40 of the 50 trials, reinforcement was provided on a constant-probability variable-interval 30-s schedule (Catania and Reynolds 1968). The levers were inserted into the chamber at the start of each trial, and were withdrawn during the intertrial interval. During the first 25 s of the trial, reinforcers were delivered only for responses on lever A, whereas during the last 25 s, reinforcers were delivered only for responses on lever B. The positions of lever A and lever B (left versus right) were counterbalanced across subjects. Four of the 50 trials in each session were probe trials, in which no reinforcers were delivered. The remaining six trials were forced-choice trials, in which only one lever was present in the chamber (lever A,

three trials; lever B, three trials). The probe and forced-choice trials were interspersed randomly among the standard trials, with the constraint that at least one standard trial occurred between successive probe or forced-choice trials. In the standard and probe trials, switching between the two levers was restricted to one switch per trial: in each trial, the first response on lever B resulted in withdrawal of lever A until the start of the next trial (Chiang et al. 1998).

3.2.4. Drug treatment

The drug treatment regimen started after 90 sessions of preliminary training under the free-operant psychophysical procedure. The drug treatment regimens were the same as in Experiment 1 (see Chapter 2).

Agonists: quipazine dimaleate, 0.5, 1, 2 mg kg⁻¹; *m*-chlorophenylbiguanide hydrochloride (*m*-CPBG), 2.5, 5, 10 mg kg⁻¹. Both drugs were dissolved in 0.9% sodium chloride solution; quipazine was administered subcutaneously 15 min before the start of the experimental session, and *m*-CPBG intraperitoneally 30 min before the start of the session.

Antagonists: topanyl 3,5-dichlorobenzoate (MDL-72222), 0.25, 0.5, 1 mg kg⁻¹ was dissolved in glacial acetic acid, buffered to pH 5.5, and diluted with 0.9% sodium chloride to give the desired concentration; it was injected intraperitoneally 30 min before the start of the session. Ketanserin tartrate, 0.5, 1, 2 mg kg⁻¹ was dissolved in 0.9% sodium chloride; it was injected subcutaneously 15 min before the start of the session.

Agonist/antagonist interaction. Quipazine 2 mg kg⁻¹ was administered alone and in combination with either MDL-72222 1 mg kg⁻¹ or ketanserin 2 mg kg⁻¹. The same vehicles and times of administration were used as in the agonist-

alone and antagonist-alone series (see above).

3.2.5. Data analysis

Only the data collected from the probe trials were used in the analysis. Separate analyses were carried out on the effects of quipazine, *m*-CPBG, MDL-72222 and ketanserin, and the interaction between quipazine, MDL-72222 and ketanserin.

Relative response rates. The mean response rate on each lever in successive time-bins was calculated for each rat under each treatment condition. Relative response rate on lever B (%B), defined as the response rate on lever B divided by the combined response rate on both levers, was analyzed by a two-factor analysis of variance (treatment \times time-bin) with repeated measures on both factors.

Psychometric functions. A two-parameter logistic function was fitted to the relative response rate data: $\%B = 100/(1+[t/T_{50}]^\epsilon)$, where t is time from trial onset, T_{50} (the indifference point) is a parameter expressing the time at which $\%B = 50\%$, and ϵ is the slope of the function (Al-Zahrani et al. 1996; ϵ has a negative value in the case of ascending sigmoid functions). The curve-fitting procedure yields estimates (\pm SE est) of the values of T_{50} and the slope, from which the Weber fraction was determined as follows. The limen was defined as half the difference between T_{75} and T_{25} (T_{75} and T_{25} are the values of t corresponding to $\%B = 75\%$ and $\%B = 25\%$), and the Weber fraction was calculated as the ratio of the limen to T_{50} . Goodness of fit of the logistic functions was expressed as the index of determination, p^2 . The values of T_{50} , ϵ , and the Weber fraction were analyzed by one-factor analyses of variance (treatments) with repeated measures. In the case of a significant effect of treatment, comparisons were made between

each active treatment and the control (vehicle alone) condition using Dunnett's test (significance criterion, $P < 0.05$). In the case of data from the quipazine-ketanserin/MDL-72222 interaction, multiple comparisons were made between treatment with quipazine alone and the combined quipazine + antagonist treatments, using Neuman-Keul's test (significance criterion, $P < 0.05$).

Overall response rates. Overall response rate was calculated for each rat under each treatment condition. For each series of treatments, the data were analyzed using a one-factor analysis of variance (treatment), with repeated measures, followed by *post-hoc* analyses as described above.

Switching. The probability of a switch occurring in each 5-s epoch of the probe trials was calculated for each rat. Logistic functions (see above) were fitted to the cumulative probability distributions, and the inflection point, S_{50} , was used as a measure of mean switching time (Body et al. 2003). The values of S_{50} were subjected to one-factor analyses of variance, as described above.

3.3. RESULTS

Under each treatment condition, response rate on lever A declined and response rate on lever B increased as a function of time from trial onset, the proportion of responding allocated to lever B (%B) increasing progressively as a function of time from trial onset.

3.3.1. Effects of the agonists

Quipazine. The effect of quipazine on relative response rate is shown in Fig. 3.1.A. Analysis of variance of these data revealed significant main effects of

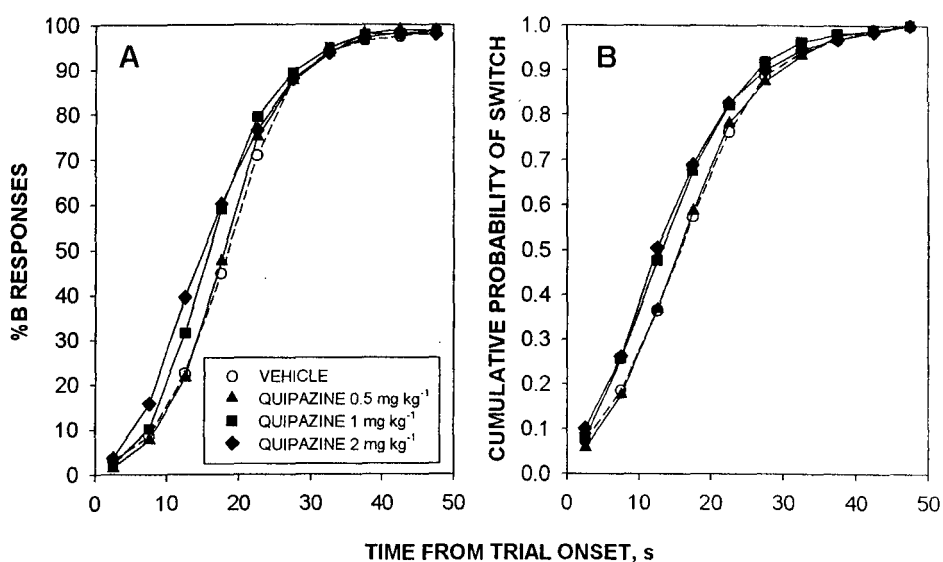


Figure 3.1 A. Effect of quipazine on relative response rate on lever B in the free-operant psychophysical procedure. *Ordinate*: percent responding on lever B (%B); *abscissa*: time from trial onset (s). Points indicate group mean data under each treatment condition (see inset). B. Effect of quipazine on probability of switching from lever A to lever B. *Ordinate*: cumulative probability of switching; *abscissa*: time from trial onset (s).

treatment [$F(3,69) = 7.4, P < 0.001$] and time-bin [$F(9,207) = 727.5, P < 0.001$], and a significant treatment \times time-bin interaction [$F(27,621) = 6.8, P < 0.001$].

Logistic functions were fitted to the data from each rat under each treatment condition; the group mean values of parameters of these functions (\pm SEM) are shown in Table 3.1. Quipazine dose-dependently displaced the psychometric function to the left, reducing the value of T_{50} ; analysis of variance showed a significant effect of treatment [$F(3,69) = 9.9, P < 0.001$], and multiple comparisons with the vehicle-alone condition indicated that quipazine 1 and 2 mg kg^{-1} significantly reduced T_{50} . Quipazine also produced some flattening of the psychometric function, this being reflected in a significant effect on the slope of the function, ϵ [$F(3,69) = 5.0, P < 0.01$], the effect of 2 mg kg^{-1} being statistically significant. The Weber fraction was increased by quipazine [$F(3,69) = 6.9, P < 0.001$], the effect of 2 mg kg^{-1} being significant.

The effect of quipazine on the cumulative probability of switching is shown in Fig.3.1B. Logistic functions were fitted to the data from each rat; group mean values of the inflection point, S_{50} (\pm SEM) are shown in Table 3.1. Quipazine reduced S_{50} [$F(3,69) = 7.7, P < 0.001$], indicating a reduction of the mean time of switching from lever A to lever B; the effects of 1 and 2 mg kg^{-1} were statistically significant.

Quipazine significantly reduced overall response rate (see Table 3.1) [$F(3,69) = 50.0, P < 0.001$], the effects of all three doses being statistically significant.

m-CPBG. The relative response rate data are shown in Fig. 3.2A. Analysis of variance showed a significant main effect of time-bin $F(9,207) = 670.8, P < 0.001$, but no significant main effect of treatment [$F(3,69) = 2.6, P > 0.05$] and

Table 3.1. Effects of the agonists on measures of performance on the free-operand psychophysical procedure: group mean values (\pm SEM)

Treatment	Relative response rate function				Cumulative switching function		Overall response rate, responses min ⁻¹
	T_{50} , s	slope, ε	p^2	Weber fraction	S_{50} , s	P^2	
Vehicle	17.8 \pm 0.8	-4.7 \pm 0.3	0.99 \pm 0.00	0.26 \pm 0.01	14.9 \pm 0.6	0.98 \pm 0.00	69.8 \pm 9.0
quipazine 0.5 mg kg ⁻¹	17.6 \pm 0.7	-4.8 \pm 0.3	0.99 \pm 0.00	0.25 \pm 0.01	14.7 \pm 0.7	0.98 \pm 0.00	61.3 \pm 9.7 *
quipazine 1 mg kg ⁻¹	15.8 \pm 0.7 *	-4.3 \pm 0.3	0.99 \pm 0.00	0.29 \pm 0.02	12.7 \pm 0.8 *	0.97 \pm 0.01	49.8 \pm 10.2 *
quipazine 2 mg kg ⁻¹	14.8 \pm 0.8 *	-3.7 \pm 0.3 *	0.98 \pm 0.00	0.35 \pm 0.03 *	12.1 \pm 0.6 *	0.97 \pm 0.00	41.0 \pm 8.2 *
Vehicle	21.3 \pm 0.7	-5.5 \pm 0.3	0.99 \pm 0.00	0.22 \pm 0.02	17.9 \pm 0.8	0.98 \pm 0.01	61.5 \pm 2.1
<i>m</i> -CPBG 2.5 mg kg ⁻¹	21.4 \pm 0.8	-6.6 \pm 0.5	0.99 \pm 0.00	0.19 \pm 0.01	18.3 \pm 0.9	0.98 \pm 0.00	62.5 \pm 1.8
<i>m</i> -CPBG 5 mg kg ⁻¹	22.6 \pm 0.8	-6.2 \pm 0.4	0.98 \pm 0.00	0.20 \pm 0.01	19.5 \pm 0.8	0.98 \pm 0.00	63.1 \pm 2.7
<i>m</i> -CPBG 10 mg kg ⁻¹	21.8 \pm 1.0	-6.4 \pm 0.6	0.98 \pm 0.01	0.21 \pm 0.02	18.2 \pm 0.9	0.96 \pm 0.01	59.4 \pm 2.1 *

Significant difference from vehicle condition, * $P < 0.05$ (see text for details)

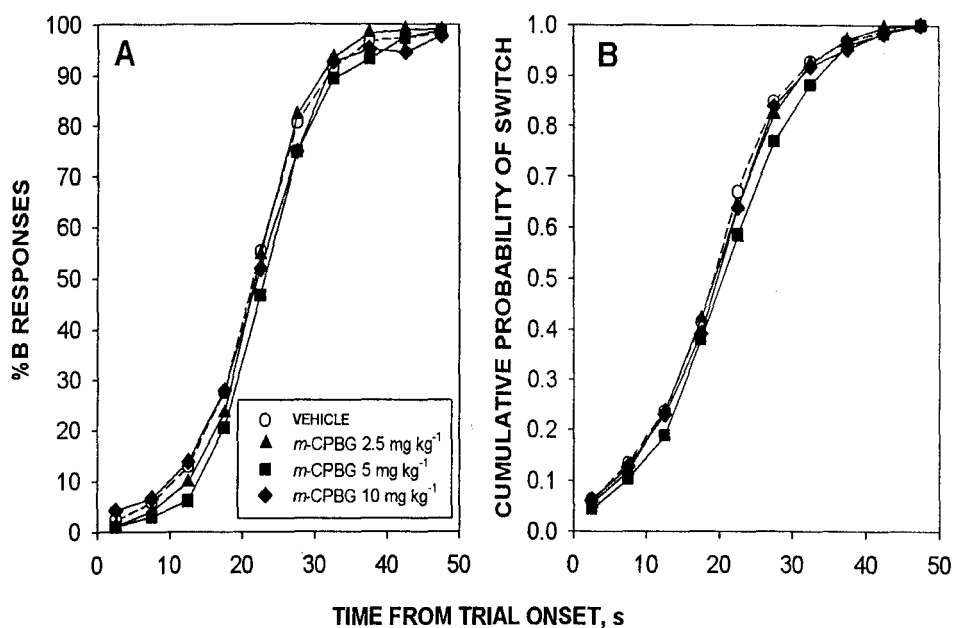


Figure 3.2 A. Effect of *m*-CPBG on relative response rate on lever B. B. Effect of *m*-CPBG on probability of switching. Conventions as in Fig. 3.1.

no significant interaction [$F(27,621) = 1.2, P>0.05$].

Parameters of the logistic functions are shown in Table 3.1. *m*-CPBG had no significant effect on the values of T_{50} [$F(3,69) = 2.2, P>0.05$], ϵ [$F(3,69) = 2.5, P>0.05$] or the Weber fraction [$F(3,69) = 2.2, P>0.05$].

Cumulative probability of switching is shown in Fig. 3.2B. The value of S_{50} was not significantly affected by *m*-CPBG [$F(3,69) = 2.1, P>0.05$] (Table 1).

Overall response rate was reduced by *m*-CPBG [$F(3,69) = 3.0, P<0.05$], the effect of 10 mg kg⁻¹ being statistically significant (Table 3.1).

3.3.2. Effects of the antagonists

MDL-72222. The relative response rate data are shown in Fig. 3.3A. Analysis of variance showed a significant main effect of time-bin [$F(9,207) = 678.1, P<0.001$], but no significant main effect of treatment [$F<1$], and no significant interaction [$F(27,621) = 1.0, P>0.05$].

Parameters of the logistic functions are shown in Table 3.2. MDL-72222 had no significant effect on the values of T_{50} [$F<1$], ϵ [$F(3,69) = 1.4, P>0.05$] or the Weber fraction [$F(3,69) = 1.3, P>0.05$].

Cumulative probability of switching is shown in Fig. 3.3B. The value of S_{50} was not significantly affected by MDL-72222 [$F<1$] (Table 3.2).

Overall response rate was increased by MDL-72222 [$F(3,69) = 6.5, P<0.05$], the effect of 0.5 mg kg⁻¹ being statistically significant (see Table 3.2).

Ketanserin. The relative response rate data are shown in Fig. 3.4A. Analysis of variance showed a significant main effect of time-bin [$F(9,207) = 524.5, P<0.001$]. The main effect of treatment was significant [$F(3,69) = 6.5,$

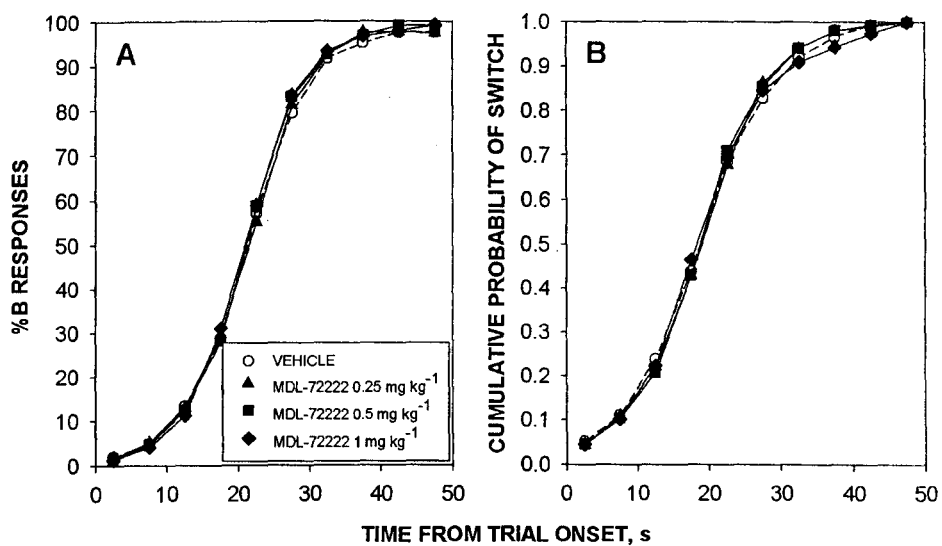


Figure 3.3 A Effect of MDL-72222 on relative response rate on lever B. B. Effect of MDL-72222 on probability of switching. Conventions as in Fig. 3.1.

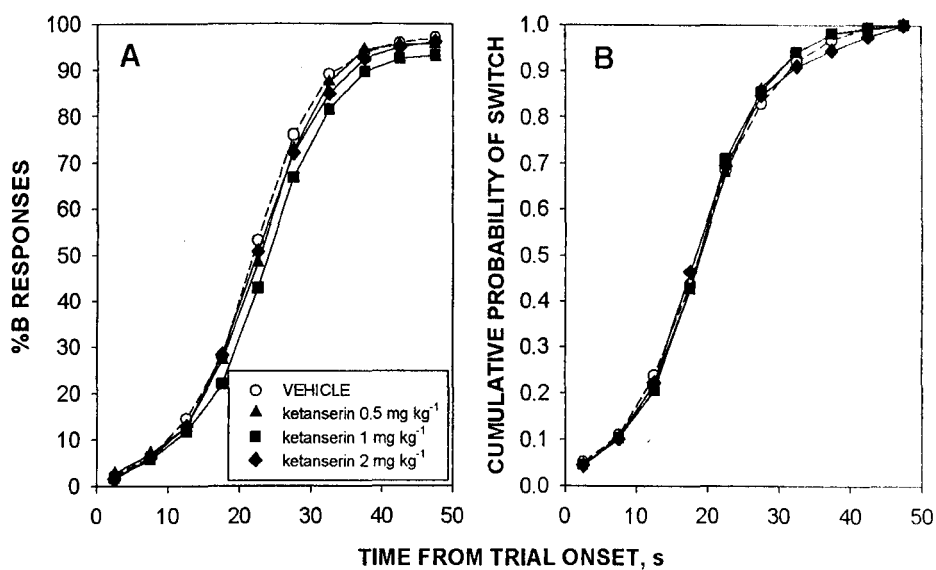


Figure 3.4 A. Effect of ketanserin on relative response rate on lever B. B. Effect of ketanserin on probability of switching. Conventions as in Fig. 3.1.

Table 3.2. Effects of the antagonists on measures of performance on free-operant psychophysical procedure: group means (\pm SEM)

Treatment	Relative response rate function				Cumulative switching function		Overall response rate, responses min ⁻¹
	T_{50} , s	slope, ϵ	p^2	Weber fraction	S_{50} , s	p^2	
Vehicle	20.8 \pm 0.8	-5.5 \pm 0.3	0.99 \pm 0.00	0.22 \pm 0.01	18.0 \pm 0.7	0.99 \pm 0.00	66.2 \pm 3.1
MDL-72222 0.25 mg kg ⁻¹	21.0 \pm 0.8	-6.3 \pm 0.6	0.99 \pm 0.00	0.20 \pm 0.02	18.1 \pm 0.6	0.98 \pm 0.00	68.1 \pm 2.9
MDL-72222 0.5 mg kg ⁻¹	20.6 \pm 0.7	-6.0 \pm 0.4	0.99 \pm 0.00	0.20 \pm 0.01	17.8 \pm 0.7	0.98 \pm 0.01	72.8 \pm 3.3 *
MDL-72222 1 mg kg ⁻¹	20.7 \pm 0.8	-6.3 \pm 0.4	0.99 \pm 0.00	0.20 \pm 0.01	17.9 \pm 0.9	0.98 \pm 0.00	67.1 \pm 3.0
Vehicle	21.5 \pm 0.9	-5.3 \pm 0.3	0.99 \pm 0.00	0.23 \pm 0.02	18.1 \pm 0.9	0.98 \pm 0.00	57.9 \pm 1.8
ketanserin 0.5 mg kg ⁻¹	22.1 \pm 1.0	-5.4 \pm 0.5	0.97 \pm 0.00	0.24 \pm 0.02	18.6 \pm 0.9	0.97 \pm 0.00	60.0 \pm 1.5
ketanserin 1 mg kg ⁻¹	23.6 \pm 1.0 *	-5.5 \pm 0.6	0.98 \pm 0.00	0.26 \pm 0.03	19.5 \pm 0.9	0.97 \pm 0.00	62.4 \pm 1.9
ketanserin 2 mg kg ⁻¹	21.9 \pm 0.9	-5.1 \pm 0.4	0.98 \pm 0.01	0.26 \pm 0.02	19.0 \pm 0.7	0.97 \pm 0.01	65.7 \pm 1.7 *

Significant difference from vehicle condition, * $P < 0.05$ (see text for details)

$P < 0.01$], but not the interaction [$F(27,621) = 1.4$, $P > 0.05$].

Parameters of the logistic functions are shown in Table 3.2. Ketanserin produced a small rightward displacement of the psychometric function, reflected in an increase in T_{50} . Analysis of variance showed a significant effect of treatment on T_{50} [$F(3,69) = 4.8$, $P < 0.01$], the 1 mg kg⁻¹ dose producing a significant increase. Ketanserin did not significantly affect the value of ϵ [$F(3,69) = 1.4$, $P > 0.05$] or the Weber fraction [$F < 1$].

Cumulative probability of switching is shown in Fig. 3.4B. The value of S_{50} was not significantly affected by ketanserin [$F(3,69) = 1.7$, $P > 0.05$] (Table 3.2). Overall response rate was increased by ketanserin [$F(3,69) = 3.3$, $P < 0.05$], the effect of 2 mg kg⁻¹ being statistically significant (see Table 3.2).

3.3.3. Agonist antagonist interaction

The relative response rate data are shown in Fig. 3.5A. Analysis of variance revealed significant main effects of time-bin [$F(9,207) = 765.1$, $P < 0.001$] and treatment [$F(3,69) = 32.2$, $P < 0.001$], and a significant treatment \times time-bin interaction [$F(27,621) = 18.9$, $P < 0.001$]. Quipazine displaced the psychometric function to the left compared to the function derived for the vehicle treatment. MDL-72222 did not appear to reverse this effect of quipazine, as the locus of the curve derived for the quipazine + MDL-72222 treatment was close to the curve derived for quipazine alone. However, ketanserin reversed the effect of quipazine, the curve derived for quipazine + ketanserin resulted lying to the right of that derived for the vehicle alone condition.

The parameters of the logistic functions are shown in Table 3.3. There was a significant effect of treatment on T_{50} [$F(3,69) = 43.3$, $P < 0.001$]. Multiple

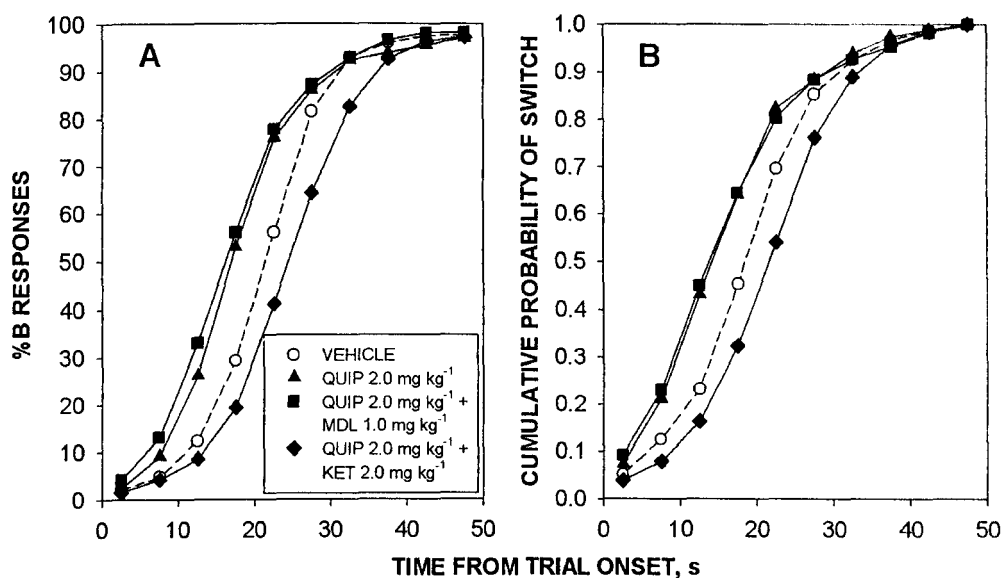


Figure 3.5. Interaction between quipazine (QUIP), MDL-7222 (MDL) and ketanserin (KET) on performance on the free-operant psychophysical procedure. **A.** Relative response rate; **B.** Probability of switching. Conventions as in Fig. 1. In both graphs, the psychometric function is displaced to the left by quipazine; this effect is reversed by co-administration of ketanserin, but not by co-administration of MDL-7222.

Table 3.3 Interaction between quipazine and the antagonists on performance on free-orient psychophysical procedure: group mean values (± SEM)

Treatment	Relative response rate function				Cumulative switching function		Overall response rate, (responses min ⁻¹)
	<i>T</i> ₅₀ , s	slope, ε	<i>p</i> ²	Weber fraction	<i>S</i> ₅₀ , s	<i>p</i> ²	
Vehicle	20.8 ±0.7	-5.7 ± 0.3	0.99 ± 0.00	0.21 ± 0.01	17.6 ± 0.6	0.98 ± 0.00	62.9 ± 2.6
quipazine 2 mg kg ⁻¹	17.0 ±0.8 *	-4.3 ± 0.3 *	0.98 ± 0.01	0.29 ± 0.02 *	13.4 ± 0.8 *	0.98 ± 0.00	40.1 ± 3.4 *
quipazine 2 mg kg ⁻¹ + MDL-72222 1 mg kg ⁻¹	15.6 ±0.8 *	-4.0 ± 0.3 *	0.98 ± 0.00	0.33 ± 0.03 *	13.1 ± 0.8 *	0.97 ± 0.01	37.8 ± 3.1 *
quipazine 2 mg kg ⁻¹ + ketanserin 2 mg kg ⁻¹	24.2 ±1.1 *#	-6.4 ± 0.3 #	0.99 ± 0.00	0.19 ± 0.01 #	20.7 ± 0.9 #	0.98 ± 0.01	68.6 ± 3.0 #

Significant difference from vehicle condition, * *P* < 0.05; significant difference from quipazine 2 mg kg⁻¹ condition, # *P* < 0.05 (see text for details)

comparisons with the vehicle condition (Dunnett's test) showed that T_{50} was significantly reduced by quipazine and by quipazine + MDL-72222 (reflecting leftward displacement of the curve), and significantly increased by quipazine + ketanserin (reflecting rightward displacement of the curve). Multiple comparisons with the quipazine condition (Newman-Keuls test) showed that the reduction in T_{50} produced by quipazine was significantly reversed by combined treatment with ketanserin but not by combined treatment with MDL-72222.

There was a significant effect of treatment on ϵ [$F(3,69) = 17.7, P < 0.001$]. Multiple comparisons with the vehicle alone condition showed that the value of ϵ was increased both by quipazine and by quipazine + MDL-72222 (reflecting flattening of the psychometric curve).

There was a significant effect of treatment on the Weber fraction [$F(3,69) = 16.2, P < 0.001$]. Multiple comparisons with the vehicle alone condition showed that quipazine and quipazine + MDL-72222 increased the Weber fraction. Multiple comparisons with the quipazine condition showed that the increase in the Weber fraction produced by quipazine was significantly reversed by ketanserin but not by MDL-72222.

There was a significant effect of treatment on overall response rate [$F(3,69) = 46.3, P < 0.001$]. Quipazine and quipazine + MDL-72222 significantly reduced overall response rate compared to the vehicle alone condition. Ketanserin, but not MDL-72222, significantly attenuated the reduction in response rate produced by quipazine.

The effect of quipazine on the cumulative probability of switching is shown in Fig. 3.5B. Logistic functions were fitted to the data from each rat; group mean values of S_{50} (\pm SEM) are shown in Table 3.3. There was a significant effect of treatment on S_{50} [$F(3,69) = 47.7, P < 0.001$]. Both quipazine and quipazine +

MDL-72222 significantly reduced S_{50} , indicating a reduction of the mean time of switching from lever A to lever B, whereas quipazine + ketanserin increased S_{50} , compared to the vehicle alone condition. The effect of quipazine was significantly reversed by ketanserin but not by MDL-72222.

3.4. DISCUSSION

In accordance with previous findings with the free-operant psychophysical procedure (Stubbs 1976; Bizo and White 1994, Chiang et al. 1998, 2000a, 2000b; Machado and Guilhardi 2000, Body et al, 2002, 2003, 2004), response rate on lever A declined, and response rate on lever B increased, during the course of the trial, this being reflected in an increasing percentage of total responding devoted to lever B (%B) as the trial progressed. The values of T_{50} and the Weber fraction derived from the relative response rate function (%B vs t), and the mean switching time, S_{50} , estimated using the cumulative probability of switching in successive epochs of the probe trials, were similar to those reported previously for rats responding under this schedule (Chiang et al. 1999, 2000a, 2000b; Body et al. 2002, 2003, 2004). Comparison of the control (vehicle-alone treatment) values of T_{50} obtained in the different phases of the experiment indicates that the value obtained during the initial assessment of quipazine was somewhat lower than the values seen in subsequent phases. This suggests that performance may not have fully stabilized when the first treatment series was initiated, despite the extensive preliminary training that the rats had received (90 daily training sessions). This does not seem to have had a major impact on the results of the experiment, as indicated by the comparable effects of quipazine in the first and last phase of the experiment (see below). Nevertheless, a longer period of preliminary training

would be desirable in future experiments.

Quipazine ($0.5\text{--}2.0\text{ mg kg}^{-1}$) produced a dose-dependent leftward displacement of the psychometric functions, reflected in significant reductions of the values of T_{50} and S_{50} . This effect indicates a facilitation of ‘premature’ switching from the lever associated with reinforcement during the first half of the trial to the lever associated with reinforcement during the latter half of the trial. The behavioural processes underlying this effect remain uncertain. One possible theoretical interpretation is that quipazine may have altered the functioning of the hypothetical internal clock that is purported to underlie interval timing behaviour (Gibbon et al. 1997; Hinton and Meck 1997). However it is important to note that this effect was not seen in the case of another timing schedule, the discrete-trials psychophysical procedure (Experiment 1: see Chapter 2). Therefore the results are not consistent with an interaction with a ‘unitary’ internal clock that, according to SET, underlies all types of interval timing performance (see Section 1.4).

Although the most conspicuous effect of quipazine was on the locus of the psychometric function, the highest dose of quipazine used in these experiments also produced some flattening of the function, and a consequent increase of the Weber fraction, suggesting that this dose of quipazine had a deleterious effect on the precision of temporal differentiation (see Gibbon et al. 1997; Killeen et al. 1997).

The selective 5-HT₃ receptor agonist *m*-CPBG (Kilpatrick et al. 1990; Dukat et al. 1996) had no significant effect on temporal differentiation. As discussed in Chapter 2 (section 2.4), it is unlikely that this reflects the use of an inadequate dose of *m*-CPBG, since the dose range used in this experiment has been found to be active in other behavioural paradigms. In doses similar to those used here, *m*-CPBG has been found to be active in behavioural tests of anxiety,

showing an ‘anxiogenic’ profile in the elevated plus maze test (Andrews and File 1992), and reversing the effect of the 5-HT₃ receptor antagonists ICS 205-930 (Nakagawa et al. 1998) and ondansetron (Eguchi et al. 2001) in other anxiety models. In the same dose range, *m*-CPBG fully substitutes for other 5-HT₃ receptor agonists in drug discrimination tests (Dukat et al. 2000). *m*-CPBG (1 and 10 mg kg⁻¹) has been found to disrupt the acquisition of conditioned responses in an autoshaping paradigm, an effect that was completely reversed by co-administration of the 5-HT₃ receptor antagonists ondansetron and tropisetron (Hong and Meneses 1996). Finally, *m*-CPBG also produces conditioned place and taste aversion; however, this effect may be mediated, at least in part, by peripheral (gastrointestinal) effects of the drug (Higgins et al. 1993).

The lack of effect of *m*-CPBG stands in contrast to the robust effect of quipazine. Quipazine has a very high affinity for 5-HT₃ receptors ($K_d = 2$ nM; Glennon et al. 1989; Sharif et al. 1991), and somewhat lower affinity (micromolar range) for several other subtypes of 5-HT receptor, including 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors (Hoyer 1988). The effect of quipazine on temporal differentiation was not altered by co-administration of MDL-72222, but was completely abolished by co-administration of ketanserin. MDL-72222 is a selective 5-HT₃ receptor antagonist (Fozard 1984); the doses used in this experiment have been shown to be behaviourally active in a variety of tests that are thought to reflect 5-HT₃ receptor function (Costall and Naylor 1992; Higgins et al. 1992; Mazzola-Pomietto et al. 1995). Taken together, these findings suggest that central 5-HT₃ receptor stimulation does not influence temporal differentiation in the rat. Furthermore, the complete reversal of quipazine’s effect on T_{50} and S_{50} by ketanserin strongly implicates 5-HT₂ receptors in this effect. Since ketanserin has an 80-100 times higher affinity for the 5-HT_{2A} receptor than for the 5-HT_{2C}

receptor (Baxter et al. 1995; Barnes and Sharp 1999), it is likely that this effects of quipazine is mediated by 5-HT_{2A} receptors (see Body et al. 2003). In this context, it may be noted that the dose of ketanserin that antagonized quipazine's effect in this experiment has previously been shown to antagonize the reduction of T_{50} and S_{50} induced by the 5-HT_{2A/2C} receptor agonist DOI (Body et al. 2003, 2004) and the 5-HT releasing agent fenfluramine (Body et al. 2004).

The effect of quipazine on temporal differentiation thus appears to be mediated not by 5-HT₃ receptors but by 5-HT_{2A} receptors. In this respect, the present results (like the results of Experiment 1) are consistent with findings with other behavioural paradigms. Although quipazine has been found to behave like other 5-HT₃ receptor agonists, including *m*-CPBG, in some tests (Dukat et al. 2000), many of its behavioural effects, including the induction of head twitching (Sanchez and Arnt 2000) and lordosis (Wolf et al. 1998), and the enhancement of progressive ratio schedule performance (Wolff and Leander 2000), are believed to be mediated by 5-HT₂ receptors. In drug discrimination studies, quipazine can substitute for both 5-HT₃ (Dukat et al. 2000) and 5-HT_{2A} (Wolff and Leander 2000; Smith et al. 2002) receptor agonists.

Quipazine produced a significant reduction of the overall rate of responding, which was reversed by co-administration of ketanserin, suggesting that this effect, like the reduction of T_{50} , was mediated by 5-HT_{2A} receptors. It is very unlikely, however, that the change in T_{50} was a direct consequence of the change in response rate. Since T_{50} is derived from relative response rate data (the percentage of overall responding devoted to lever B), it should be impervious to changes in absolute response rate (Chiang et al, 2000a; Odum et al, 2002). Moreover, the other index of the indifference point, the mean switching time S_{50} , which is not calculated from response rates, was affected by quipazine in the same

manner as T_{50} . It may also be noted that previous experiments have failed to identify consistent correlations between changes in T_{50} and changes in overall response rate, either within or between drug conditions (Body et al. 2004). Whether the effects of DOI and quipazine on temporal differentiation and overall response rate are mediated by different populations of 5-HT_{2A} receptors is a question for future experiments.

Taken together, the present findings that a selective 5-HT₃ receptor agonist, *m*-CPBG, was without effect, and that the effect of a mixed 5-HT_{2/3} receptor agonist, quipazine, was fully attributable to its action at 5-HT_{2A} receptors, suggest that while 5-HT_{2A} receptor stimulation has a robust influence on temporal differentiation, 5-HT₃ receptor stimulation does not. This conclusion may have implications for current conceptions of the role of 5-HT/dopamine interactions in interval timing behaviour. It is widely believed that dopaminergic transmission in the basal ganglia plays a pivotal role in the regulation of interval timing (Meck 1986, 1996; Gibbon et al. 1997; Matell and Meck 2000; see Section 1.5.1.3). There is good evidence that 5-HT₃ receptors contribute to the regulation of dopamine release (Blandina et al. 1989; Carboni et al. 1989; Zazpe et al. 1994; Cerrito et al. 1996). It might therefore be expected that 5-HT₃ receptor stimulation would alter timing performance, an expectation that found no support in the present experiments. The explanation for the apparent discrepancy may lie in regional differences in the nature of 5-HT/dopamine interactions. There is evidence that the influence of 5-HT₃ receptors on dopamine release is mainly confined to structures innervated by the mesolimbic/mesocortical dopaminergic projection (including the ventral striatum/nucleus accumbens), rather than the dorsal striatum which receives its input from the nigrostriatal pathway (Wang et al. 1998; DeDeurwaerdere et al. 1998; Porras et al. 2003). The failure of 5-HT₃ receptor

stimulation to affect temporal differentiation may therefore reflect a primary involvement of the dorsal rather than the ventral striatal dopaminergic mechanisms in this behaviour (Hinton and Meck 1997; Matell and Meck 2000).

The location of the 5-HT₂ receptors that apparently mediate not only quipazine's effects on temporal differentiation, but also those of DOI (Body et al. 2003, 2004) and fenfluramine (Body et al. 2004) remains uncertain. There is evidence for a facilitatory role of striatal 5-HT_{2A} and 5-HT_{2C} receptors on dopamine release in this structure (DiGiovanni et al. 1999; Lucas and Spampinato 2000). Further experiments using intra-striatal injection of selective 5-HT_{2A} and 5-HT_{2C} receptor agonists may help to establish whether these receptor populations are responsible for the disruption of temporal differentiation induced by 5-HT₂ receptor agonists.

Finally, it should be noted that, while pharmacological stimulation of 5-HT_{2A} receptors has been found to exert consistent effects on temporal differentiation (Body et al. 2003, 2004; present results), it is unlikely that endogenous stimulation of this receptor population makes a major contribution to temporal differentiation under normal conditions. If this were the case, blockade of 5-HT_{2A} receptors would be expected to produce a rightward displacement of the psychometric function (i.e. the opposite effect to that produced by 5-HT_{2A} receptor agonists: Body et al. 2003, 2004). Although there is some indication that this occurred in the present experiment (see, especially, Figure 3.5), the results of previous experiments indicate that this is not a reliable effect of ketanserin (see Body et al. 2003, 2004). It should also be emphasized that even after complete destruction of the ascending 5-HTergic pathways, rats are still able to execute accurate temporal differentiation performance on the free-operant psychophysical procedure (Chiang et al. 1999; Body et al. 2002, 2004).

CHAPTER 4

Experiment 3:

EFFECTS OF 2,5-DIMETHOXY-4- IODOAMPHETAMINE (DOI) ON TEMPORAL DISCRIMINATION

4.1. INTRODUCTION

As reviewed in Chapter 2, two major types of timing schedule are *immediate* and *retrospective* timing schedules (Killeen and Fetterman, 1988; Killeen et al. 1997). Immediate timing schedules require the organism to regulate its own behaviour in time (*temporal differentiation*), whereas retrospective timing tasks entail discrimination of the durations of exteroceptive stimuli (*temporal discrimination*). Examples of the two types of timing are free-operant and discrete-trials psychophysical procedures (Stubbs 1976; Body et al. 2002) (for a full description see Section 1.2.1.).

In a recent series of experiments, Body et al. (2003, 2004) obtained evidence that 5-HT₂ receptor stimulation disrupts temporal differentiation in the free-operant psychophysical procedure. The 5-HT_{2A/2C} receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) dose-dependently reduced T_{50} in this schedule (Body et al. 2003, 2004). This effect of DOI was mimicked by the 5-HT releasing agent fenfluramine, the effects of both drugs being abolished by the 5-HT_{2A} receptor antagonist ketanserin (Body et al. 2004).

The experiment described in Chapter 3 of this thesis (Experiment 2) extended these observations. It was found that quipazine, a non-selective 5-HT receptor agonist with high affinity for both 5-HT₃ and 5-HT_{2A} receptors, also reduced T_{50} in the free-operant psychophysical procedure, an effect that was antagonized by ketanserin, but not by the selective 5-HT₃ receptor antagonist topamyl 3,5-dichlorobenzoate (MDL-72222), implicating 5-HT_{2A} rather than 5-HT₃ receptors in this effect.

Experiment 1, described in Chapter 2, showed that quipazine also affected temporal discrimination in the discrete-trial psychophysical procedure. However,

instead of reducing T_{50} , as it did in the free-operant psychophysical procedure, quipazine flattened the psychometric function in the discrete-trials psychophysical schedule, increasing the Weber fraction; it also displaced the function to the right, increasing T_{50} . These effects of quipazine were reversed by ketanserin, suggest that they were mediated by 5-HT_{2A} receptors. The experiment described in this chapter was carried out in order to obtain further information on the effect of 5-HT_{2A} receptor stimulation on temporal discrimination. The aims of the experiment were firstly to examine the effect of DOI on performance on the discrete-trials psychophysical procedure, and secondly to examine the sensitivity of DOI's effect to ketanserin and the highly selective 5-HT_{2A} receptor antagonist (±)2,3-dimethoxyphenyl-1-(2-(4-piperidine)-methanol) (MDL-100907) (see Barnes and Sharp 1999; Hoyer et al. 2002; Leysen 2004).

4.2. METHODS

4.2.1. Subjects

Twenty female Wistar rats, aged approximately 4 months and weighing 250-290 g at the start of the experiment, were housed under the same conditions as those used in Experiment 1.

4.2.2. Apparatus

The rats were trained in operant conditioning chambers (Campden Instruments Limited, Sileby, UK), similar to those used in Experiment 1 (see Section 2.2.2., for description). The only difference between the chambers used in Experiment 1

and those used in the present experiment was that in the present experiment the reinforcer delivering device consisted of a motor-operated pellet dispenser which delivered 45-mg food pellets.

4.2.3. Behavioural training

At the start of the experiment, the food-deprivation regimen was started and the rats were gradually reduced to 80% of their free-feeding body weights. They were then trained to press the levers, and were exposed to a discrete-trials continuous reinforcement schedule, in which the two levers were presented in random sequence, for three sessions. Thereafter, the rats underwent 50-minute training sessions under the discrete-trials psychophysical procedure identical to that described earlier (see Section 2.2.3.).

4.2.4. Drug treatment

The drug treatment regimen started after 90 sessions of preliminary training under the discrete-trials psychophysical procedure. Injections of drugs were given on Tuesdays and Fridays, and injections of the vehicle alone (0.9% sodium chloride solution) on Mondays and Thursdays; no injections were given on Wednesdays, Saturdays or Sundays. Each dose of each drug was administered five times, in order to accrue a sufficient number of trials for function fitting (see below). The order of treatments within each series was balanced within and between animals according to a Latin square. Subcutaneous injections were given using a 26-gauge needle at a volume of 1.0 ml kg⁻¹; intraperitoneal injections were given using a 25-gauge needle at a volume of 2.5 ml kg⁻¹. The doses of the drugs tested were as

follows: 2,5,-dimethoxy-4-iodoamphetamine (DOI), 0.0625, 0.125, 0.25 mg kg⁻¹ (s.c.); ketanserin 2 mg kg⁻¹ (s.c.); (±)2,3-dimethoxyphenyl-1-(2-(4-piperidine)-methanol) (MDL-100907), 0.5, 1 mg kg⁻¹ (i.p.). DOI and ketanserin were dissolved in 0.9% sodium chloride solution. MDL 100907 was dissolved in glacial acetic acid and sterile water, buffered to pH 5.5 and diluted to volume with 0.9% sodium chloride solution. DOI and ketanserin were administered 15 minutes, and MDL-100907 25 minutes before the experimental session.

4.2.5. **Data analysis**

Separate analyses were carried out on the each treatment series (see below).

For each treatment, the percentages of responses emitted on lever B (%B) at each time-point were analysed by two-factor analyses of variance (treatment × time) with repeated measures on both factors. In the event of a significant main effect of treatment or a significant treatment × time interaction, analyses of the simple main effect at each time-point were carried out, followed by comparisons between each active treatment with the control (vehicle-alone) condition using Dunnett's test. In the case of data from the drug interaction studies, multiple comparisons were made between treatment with DOI alone and the combined DOI + antagonist treatments, using Neuman-Keul's test.

Quantitative analysis of the psychometric functions was identical to that carried out in Experiment 1 (see Section 2.2.5.)

4.3. **RESULTS**

Under each treatment condition, the proportion of responding allocated to lever B

(%B) increased progressively as a function of stimulus duration, t . Under the vehicle-alone condition and all active treatment conditions, the number of 'missed' trials (i.e. trials in which no response was emitted on either lever A or lever B) was <0.5%.

4.3.1. Effect of DOI

The effect of DOI (0.0625, 0.125, 0.25 mg kg⁻¹) on proportional choice of lever B (%B) is shown in Figure 4.1. Analysis of variance revealed that the main effect of treatment was not statistically significant [$F(3,57) = 2.6, P=0.06$]. The main effect of time was significant [$F(9,171) = 551.0, P<0.001$], and there was a significant treatment \times time interaction [$F(27,513) = 2.9, P<0.001$]. Analysis of the simple main effects revealed significant treatment effects 12.5 s after trial onset, and at all time points >25 s after trial onset. Multiple comparisons with vehicle showed that DOI 0.25 mg kg⁻¹ produced a significant increase in %B 12.5 s after trial onset and significant decreases 32.5, 37.5, 42.5 and 47.5 s after trial onset.

Logistic functions were fitted to the data from each rat under each treatment condition; the group mean values of parameters of these functions (\pm SEM) are shown in Table 4.1. DOI flattened the psychometric function and tended to displace it rightwards; these effects are reflected in the parameter values. There was an apparent dose-dependent increase in the value of T_{50} ; however, analysis of variance did not reveal a significant effect of treatment [$F(3,57) = 1.7, \text{N.S.}$]. There was a significant effect on the slope, ϵ [$F(3,57) = 12.4, P<0.001$], the increase in ϵ produced by DOI 0.125 and 0.25 mg kg⁻¹ being statistically significant. The Weber fraction was increased by DOI [$F(3,57) = 10.4, P<0.001$], the effect of 0.25 mg kg⁻¹ being significant.

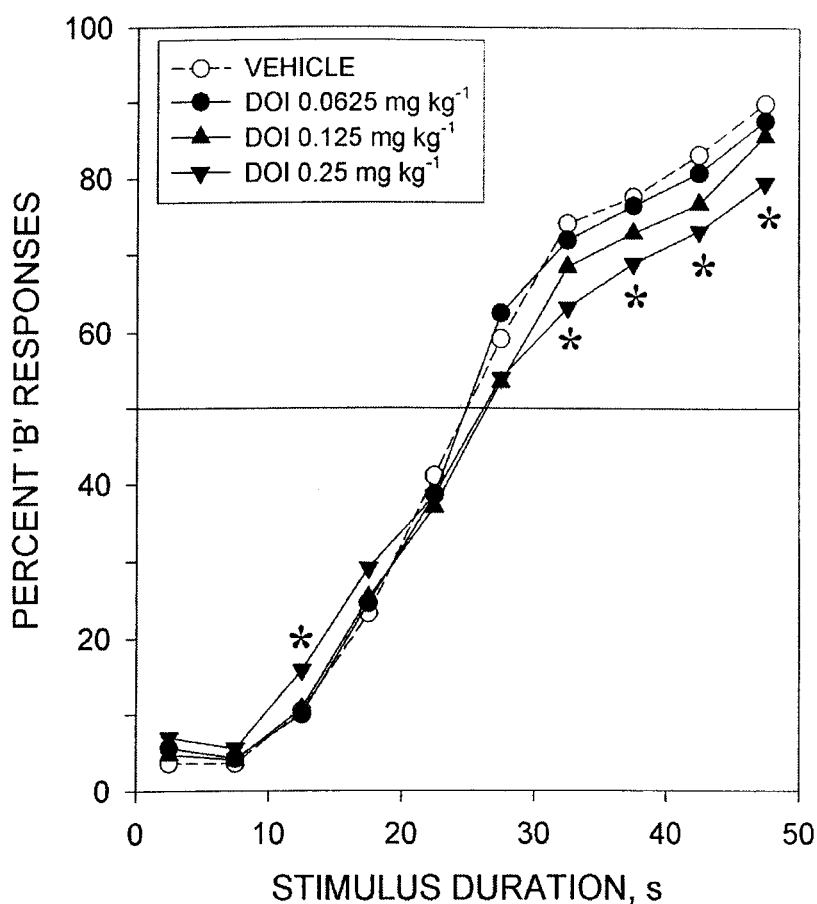


Figure 4.1. Effect of DOI (0.0625, 0.125, 0.25 mg kg⁻¹) on relationship between proportional choice of lever B (%B) and stimulus duration (*t*, seconds) in the discrete-trials psychophysical procedure. Points indicate group mean data under each treatment condition (see inset). Significance of difference from vehicle-alone treatment: * *P* < 0.05.

Table 4.1. Effects of the DOI on measures of performance on the discrete-trials psychophysical procedure: group mean values (\pm SEM)

Treatment	Parameters of logistic psychometric function			
	T_{50} , s	slope, ε	p^2	Weber fraction
Vehicle	25.2 \pm 0.6	-3.4 \pm 0.1	0.98 \pm 0.01	0.34 \pm 0.02
DOI 0.0625 mg kg ⁻¹	25.4 \pm 0.7	-3.3 \pm 0.2	0.93 \pm 0.01	0.37 \pm 0.03
DOI 0.125 mg kg ⁻¹	27.0 \pm 1.3	-3.0 \pm 0.2 *	0.93 \pm 0.02	0.40 \pm 0.02
DOI 0.25 mg kg ⁻¹	28.1 \pm 2.0	-2.5 \pm 0.2 *	0.89 \pm 0.02	0.53 \pm 0.05 *

* Significantly different from vehicle condition, $P < 0.05$ (see text for details)

4.3.2. Interaction between DOI and ketanserin

The %B data are shown in Figure 4.2. Analysis of variance revealed significant main effects of treatment [$F(3,57) = 5.1, P < 0.01$] and time [$F(9,171) = 579.8, P < 0.001$], and a significant treatment \times time interaction [$F(27,513) = 4.03, P < 0.001$]. Analysis of the simple main effects revealed significant treatment effects 7.5 s after trial onset, and at all time points ≥ 32.5 s after trial onset. Multiple comparisons with the vehicle-alone condition showed that DOI produced a significant increase in %B 7.5 s after trial onset and significant decreases in %B 32.5, 37.5, 42.5 and 47.5 s after trial onset. In no case did DOI + ketanserin differ significantly from vehicle-alone treatment, and in every case DOI + ketanserin differed significantly from the DOI-alone treatment conditions.

The parameters of the logistic functions are shown in Table 4.2. There was a significant effect of treatment on T_{50} [$F(3,57) = 3.8, P < 0.02$]. Multiple comparisons with the vehicle condition showed that T_{50} was significantly increased by DOI, but not significantly changed by ketanserin or DOI + ketanserin. Multiple comparisons with the DOI condition showed that the increase in T_{50} produced by DOI was significantly reversed by combined treatment with ketanserin.

There was a significant effect of treatment on ϵ [$F(3,57) = 11.5, P < 0.001$]. Multiple comparisons with the vehicle alone condition showed that the value of ϵ was increased by DOI, but not by ketanserin or by DOI + ketanserin. Multiple comparisons with the DOI condition showed that DOI's effect on the parameter was reversed by ketanserin.

There was a significant effect of treatment on the Weber fraction [$F(3,57) = 9.6, P < 0.001$]. Multiple comparisons with the vehicle alone condition showed

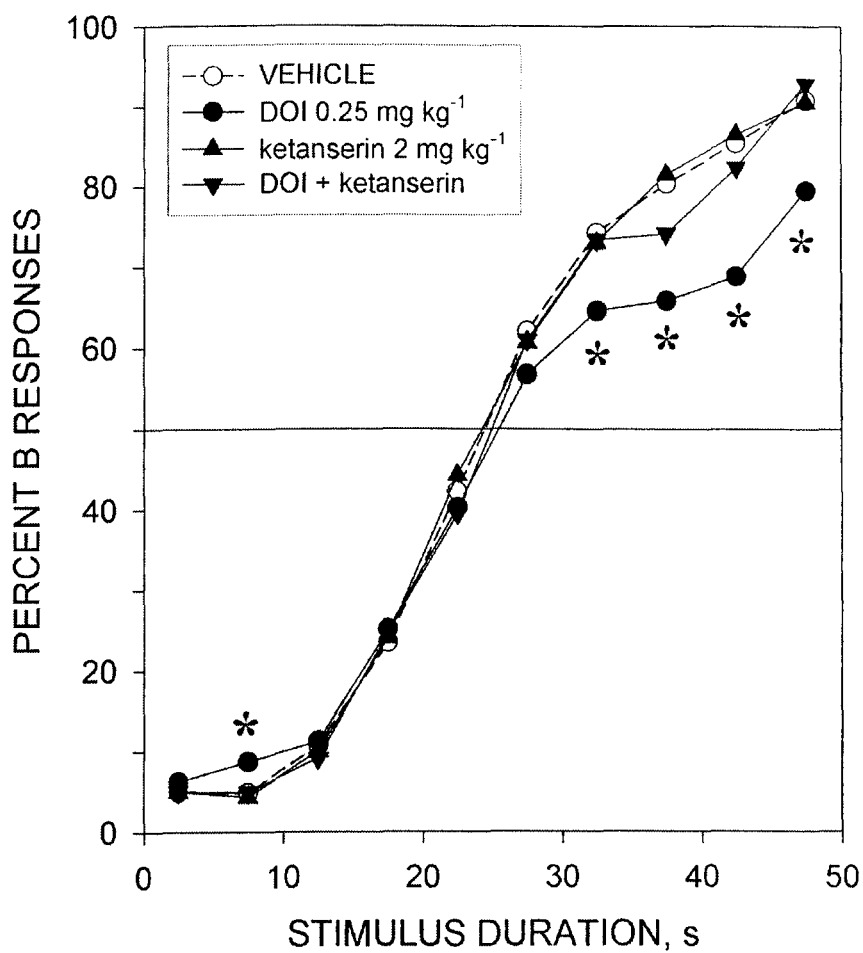


Figure 4.2. Interaction between ketanserin (2 mg kg⁻¹) and DOI (0.25 mg kg⁻¹) on relationship between proportional choice of lever B and stimulus duration in the discrete-trials psychophysical procedure. Conventions as in Fig. 4.1.

Table 4.2. Interaction between DOI and ketanserin on measures of performance on the discrete-trials psychophysical procedure: group mean values (\pm SEM)

Treatment	Parameters of logistic psychometric function			
	T_{50}, s	slope, ϵ	p^2	Weber fraction
Vehicle	24.5 \pm 0.6	-3.7 \pm 0.3	0.98 \pm 0.00	0.34 \pm 0.03
DOI 0.25 mg kg ⁻¹	28.2 \pm 1.5*	-2.5 \pm 0.2*	0.89 \pm 0.02	0.53 \pm 0.05*
ketanserin 2 mg kg ⁻¹	24.6 \pm 0.7	-3.7 \pm 0.2	0.95 \pm 0.01	0.33 \pm 0.03
DOI 0.25 mg kg ⁻¹ + ketanserin 2 mg kg ⁻¹	25.0 \pm 0.8#	-3.6 \pm 0.3#	0.94 \pm 0.01	0.36 \pm 0.04#

that the Weber fraction was increased by DOI, but not by ketanserin or by DOI + ketanserin. Multiple comparisons with the DOI condition showed that the increase in the Weber fraction produced by DOI was significantly reversed by ketanserin.

4.3.3. Interaction between DOI and MDL-100907

Two treatment series were carried out, examining the effects of MDL-100907 0.5 and 1.0 mg kg⁻¹, respectively. The %B data from the two series are shown in Figs. 4.3A and 4.3B. Analysis of variance of these data showed that in the first series (MDL-100907 0.5 mg kg⁻¹), there were significant main effects of treatment [$F(3,57) = 4.8, P < 0.01$] and time [$F(9,171) = 500.6, P < 0.001$], and a significant treatment \times time interaction [$F(27,621) = 4.4, P < 0.001$]. Analysis of the simple main effects revealed significant treatment effects 27.5, 37.5, 42.5 and 47.5 s after trial onset. Multiple comparisons with the vehicle-alone condition showed that DOI produced significant decreases in %B at all these time points. Multiple comparisons between the DOI and DOI + MDL-100907 (0.5 mg kg⁻¹) conditions indicated that MDL-100907 significantly attenuated the effect of DOI only at the 47.5-s time point. In the second series (MDL-100907 1.0 mg kg⁻¹), there were significant main effects of treatment [$F(3,57) = 10.9, P < 0.001$] and time [$F(9,171) = 553.2, P < 0.001$], and a significant treatment \times time interaction [$F(27,621) = 5.1, P < 0.001$]. Analysis of the simple main effects revealed significant treatment effects at all time points ≥ 22.5 s after trial onset. Multiple comparisons with the vehicle-alone condition showed that DOI produced significant decreases in %B at all these time points. Multiple comparisons between the DOI and DOI + MDL-100907 (1.0 mg kg⁻¹) conditions indicated that MDL-100907 significantly attenuated the effect of DOI at all these time points except 37.5 s after trial onset.

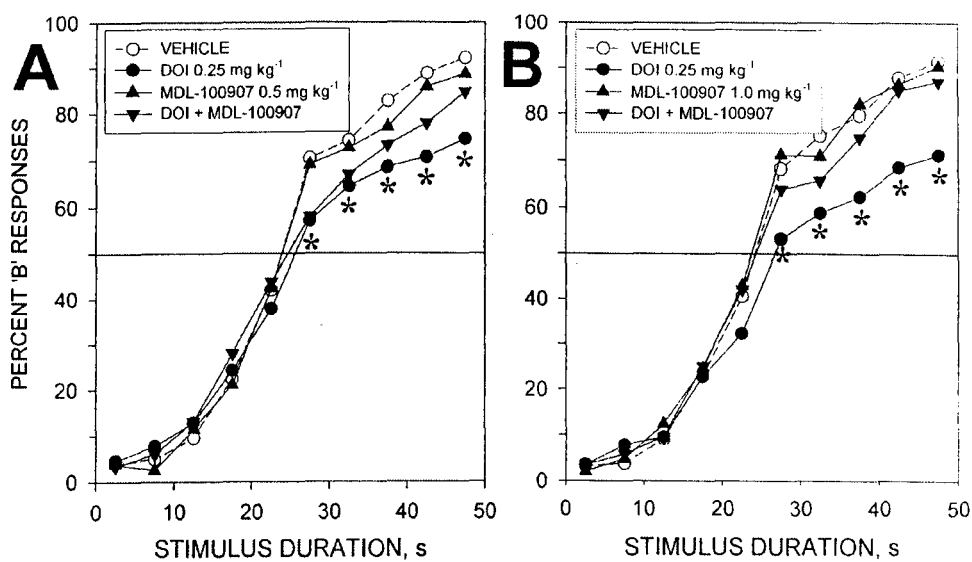


Figure 4.3. Interaction between MDL-100907 (0.5 mg kg⁻¹: **A**; 1.0 mg kg⁻¹: **B**) and DOI (0.25 mg kg⁻¹) on relationship between proportional choice of lever B and stimulus duration in the discrete-trials psychophysical procedure. Conventions as in Fig. 4.1.

The parameters of the logistic functions are shown in Table 3. In the first series, there was a significant effect of treatment on T_{50} [$F(3,57) = 3.5, P < 0.02$]. Multiple comparisons with the vehicle condition (Dunnett's test) showed that T_{50} was significantly increased by DOI, but not significantly changed by the other treatments. Multiple comparisons with the DOI condition showed that the increase in T_{50} produced by DOI was significantly reversed by combined treatment with MDL-100907 0.5 mg kg⁻¹. In the second series, there was also a significant effect of treatment on T_{50} [$F(3,57) = 10.8, P < 0.001$]. Again, DOI significantly increased T_{50} , compared to the vehicle condition, the other treatment conditions having no significant effect. There was a significant difference between the DOI and DOI + MDL-100907 1.0 mg kg⁻¹ conditions, indicating that MDL-100907 significantly reversed the effect of DOI on T_{50} .

There were significant effects of treatment on ϵ in both treatment series [first series: $F(3,57) = 11.1$; second series: $F(3,57) = 13.1$; $P < 0.001$ in both cases]. Multiple comparisons indicated that, in both cases, DOI significantly increased this parameter, compared to the vehicle alone condition, whereas neither dose of MDL-100907 had a significant effect. The effect of DOI on ϵ was reversed by MDL-100907 1.0 mg kg⁻¹ but not by MDL-100907 0.5 mg kg⁻¹.

There were significant effects of treatment on the Weber fraction [first series: $F(3,57) = 5.9$; second series: $F(3,57) = 10.8$; $P < 0.05$ in both cases]. Multiple comparisons indicated that, in both cases, DOI significantly increased the Weber fraction, compared to the vehicle alone condition, whereas neither dose of MDL-100907 had a significant effect. The effect of DOI on the Weber fraction was reversed by MDL-100907 1.0 mg kg⁻¹ but not by MDL-100907 0.5 mg kg⁻¹.

Table 4.3. Interaction between DOI and MDL-100907 on measures of performance on the discrete-trials psychophysical procedure: group mean values (\pm SEM)

Treatment	Parameters of logistic psychometric function			
	T_{50} , s	slope, ϵ	p^2	Weber fraction
Vehicle	24.1 \pm 0.7	-4.1 \pm 0.3	0.97 \pm 0.01	0.31 \pm 0.03
DOI 0.25 mg kg ⁻¹	28.8 \pm 1.7*	-2.5 \pm 0.2*	0.86 \pm 0.05	0.63 \pm 0.11*
MDL-100907 0.5 mg kg ⁻¹	24.8 \pm 1.3	-4.1 \pm 0.4	0.95 \pm 0.01	0.33 \pm 0.03
DOI 0.25 mg kg ⁻¹ + MDL-100907 0.5 mg kg ⁻¹	25.3 \pm 1.1#	-2.8 \pm 0.2*	0.93 \pm 0.01	0.47 \pm 0.05
Vehicle	24.3 \pm 0.6	-3.9 \pm 0.3	0.95 \pm 0.01	0.32 \pm 0.03
DOI 0.25 mg kg ⁻¹	31.7 \pm 2.0*	-2.6 \pm 0.2*	0.85 \pm 0.05	0.63 \pm 0.16*
MDL-100907 1.0 mg kg ⁻¹	24.0 \pm 0.9	-3.8 \pm 0.3	0.94 \pm 0.02	0.37 \pm 0.06
DOI 0.25 mg kg ⁻¹ + MDL-100907 1.0 mg kg ⁻¹	25.0 \pm 0.9#	-3.2 \pm 0.2#	0.94 \pm 0.01	0.40 \pm 0.04#

* Significantly different from vehicle condition, $P < 0.05$; # significantly different from DOI 0.25 mg kg⁻¹, $P < 0.05$

4.4. DISCUSSION

Temporal discrimination performance in the discrete-trials psychophysical procedure used in this experiment was similar to that reported previously: proportional choice of lever B increased as a sigmoid function of stimulus duration, this being well described by a two-parameter logistic equation (Body et al. 2002; Also see chapter 2).

DOI produced a dose-dependent disruption of temporal discrimination, which was most readily apparent in the case of longer stimulus durations. This resulted in rightward displacement and flattening of the psychometric function, reflected in a trend for the value of T_{50} to be increased (statistically significant in three of the four treatment series), combined with increases in the slope parameter, ϵ , and the Weber fraction.

The increase in ϵ and the Weber fraction are indicative of an impairment of the precision with which the rats discriminated the durations of the light stimulus (see Killeen et al. 1997). It is not possible to say with certainty, on the basis of these results, whether the deleterious effect of DOI on discriminative accuracy is specific for the temporal dimension, or whether it may reflect a more general breakdown of stimulus control. Further experiments, examining the effect of DOI on discrimination along other stimulus dimensions will be needed to address this question.

The increase in T_{50} induced by DOI is open to more than one interpretation. One possibility is that DOI may have had a direct effect on the neural mechanisms of timing. For example, according to pacemaker-based models of timing, an acute rightward shift of the psychometric function may reflect an increase in the period of the hypothetical pacemaker (see Gibbon 1991; Gibbon et al. 1997a; Hinton and Meck 1997). However, another possibility, discussed in

Chapter 2, in the context of quipazine's effect on T_{50} , is that the rightward shift of the psychometric function, like the increase in the Weber fraction, may reflect a breakdown of stimulus control. Inspection of the psychometric functions shown in Figures 4.1-4.3 indicates that DOI had relatively little effect on discriminative accuracy in the case of shorter stimulus durations, but markedly reduced the accuracy of discrimination of longer intervals. It is possible that stimulus control is relatively weak, and therefore more vulnerable to disruption, in the case of longer durations (see Section 2.4.).

The pattern of effect of DOI seen in this experiment is quite unlike that seen in experiments that have employed other types of timing schedule. In contrast to increase of T_{50} seen in the retrospective timing schedule used in this experiment, DOI has been found to produce leftward displacement of the timing function in immediate timing tasks. Thus, Body et al. (2003, 2004a) found that DOI dose-dependently reduced T_{50} in the free-operant psychophysical procedure (Stubbs 1976), and as will be discussed in another chapter of this thesis, it was found that DOI displaced the response rate function to the left, reducing the peak time, in the fixed-interval peak procedure (Catania 1970; Roberts 1981). It is difficult to see how these very different effects of DOI on temporal discrimination and temporal differentiation can both be accounted for in terms of an interaction with a unitary pacemaker that is purported to underlie both types of timing behaviour (Gibbon 1977, 1991), since the increase and decrease of T_{50} would seem to imply both a lengthening and a shortening of the period of the pacemaker. A possible solution to this problem is suggested by recent interpretations of the effects of amphetamine-like drugs on interval timing. Meck and Benson (2002) and Buhusi (2003) have proposed that these drugs may alter timing performance by two separate mechanisms: a direct interaction with the hypothetical pacemaker,

and disruption of ‘attention-sharing’. It remains to be seen whether the effects of DOI on timing are amenable to a similar interpretation. However it may be noted that any such attempt to account for DOI’s effects on timing on the basis of two distinct processes must be predicated on an explanation of why these processes make different relative contributions to temporal discrimination and temporal differentiation performance.

The effect of DOI on temporal discrimination seen in this experiment is similar to the effect of quipazine seen in Experiment 1 (see Section 2.3). Quipazine has nanomolar affinity for 5-HT₃ receptors, and somewhat lower affinity for 5-HT₂ receptors (Hoyer 1988; Glennon et al., 1989; Sharif et al., 1991). 5-HT₃ receptors appeared not to be involved in quipazine’s effect on temporal discrimination, since the effect could not be reversed by the selective 5-HT₃ receptor antagonist MDL-72222 (see Section 2.3). However, quipazine’s effect was completely reversed by the 5-HT₂ receptor antagonist ketanserin. Since ketanserin has approximately 80-100-fold higher affinity for 5-HT_{2A} than for 5-HT_{2C} receptors (Baxter et al. 1995; Barnes and Sharp 1999), it was suggested that quipazine’s effect on temporal discrimination was probably mediated by 5-HT_{2A} receptors (see Section 2.4).

DOI is a full agonist at both 5-HT_{2A} and 5-HT_{2C} receptors (see Hoyer et al. 2002). In view of ketanserin’s preference for 5-HT_{2A} receptor over the 5-HT_{2C} receptor, the ability of this antagonist to reverse DOI’s effect on temporal discrimination in the present experiment suggests a predominant involvement of 5-HT_{2A} receptors. However, more persuasive evidence for this suggestion is provided by the ability of MDL-100907 to reverse DOI’s effect, because MDL-100907 is a highly selective 5-HT_{2A} receptor antagonist with minimal affinity for 5-HT_{2C} receptors (see Barnes and Sharp 1999; Hoyer et al. 2002; Leysen 2004).

In conclusion, the present results, taken together with those of experiment 1, indicate that 5-HT_{2A} receptor stimulation disrupts temporal discrimination in the rat. However, the behavioural mechanisms that underlie this effect remain to be clarified.

CHAPTER 5

Experiment 4:

EFFECTS OF STIMULATION OF 5-HT₂ RECEPTORS IN THE DORSAL STRIATUM ON TEMPORAL DISCRIMINATION

5.1 INTRODUCTION

The experiments presented in Chapters 2 and 4 (Experiments 1 and 3) provide evidence for a disruptive effect of 5-HT_{2A} receptor stimulation on temporal discrimination. Experiment 1 showed that quipazine, a non-selective 5-HT receptor agonist with high affinity for 5-HT₃ receptors and somewhat lower affinity for 5-HT_{2A} receptors, flattened the psychometric timing function in the discrete-trials psychophysical procedure, increasing the Weber fraction. This effect was evidently not mediated by 5-HT₃ receptors, because it was resistant to the selective 5-HT₃ receptor antagonist MDL-72222. The ability of ketanserin, a 5-HT₂ receptor antagonist with higher affinity for 5-HT_{2A} receptors than for other subtypes of 5-HT₂ receptor, to reverse this effect of quipazine, strongly implicated 5-HT_{2A} receptors in the effect. Experiment 3 extended these observations by showing that the 5-HT_{2A/2C} receptor agonist DOI had a similar disruptive effect on temporal discrimination to quipazine, and that DOI's effect was reversed both by ketanserin and by the highly selective 5-HT_{2A} receptor antagonist MDL-100907.

The anatomical location of the 5-HT_{2A} receptors that mediate these effects on temporal discrimination remains unknown. 5-HT_{2A} receptors are widely distributed in the brain, the densest populations being found in the basal ganglia and cerebral cortex (Barnes and Sharp 1999; Hoyer et al. 2002; Leysen 2004). The experiments described in this chapter examined the possibility that the 5-HT_{2A} receptor population relevant to temporal discrimination may be located in the dorsal striatum. There is a great deal of evidence that the dorsal striatum plays a major role in voluntary timing behaviour (Gibbon et al. 1997; Hinton and Meck 1997, 2004; Harrington et al. 1998; Meck and Benson 2001; Ferrandez et al. 2003; Matell et al. 2003; Nenadic et al. 2003; Pastor et al. 2004; Meck 2005; see also

Section 1.4.5), and the presence of a dense population of 5-HT_{2A} receptors in this structure suggests that this may be an appropriate starting point for a search for the location of the 5-HT_{2A} receptors that mediate effects on temporal discrimination.

The main objectives of the present experiments were firstly to examine the effect of intra-striatal injection of DOI and MDL100907 on temporal discrimination, and secondly to examine whether the effect of systemically administered DOI on temporal discrimination would be blocked either by the highly selective 5-HT_{2A} receptor antagonist MDL-100907 (see Barnes and Sharp 1999; Hoyer et al. 2002), or by the highly selective 5-HT_{2C} receptor antagonist RS-102221 (Bonhaus et al. 1997), administered directly into the dorsal striatum.

5.2. METHODS

5.2.1. Subjects

Twenty nine female Wistar rats aged approximately 4 months and weighing 250-290 g at the start of the experiment were housed under the same condition as in Experiment 1 (see Section 2.2.1)

5.2.2. Apparatus

The rats were trained in operant conditioning chambers (CeNeS Ltd, Cambridge, UK) of internal dimensions 25 cm × 25 cm × 22 cm. One wall of chamber contained a recess fitted with a hinged Perspex flap, into which a peristaltic pump could dispense the liquid reinforcer (0.6 M sucrose solution). In other respects, the chambers were similar to those used in the previous experiments (see Section

2.2.2.).

5.2.3. Behavioural training

At the start of the experiment, the food deprivation regimen commenced and the rats were gradually reduced to 80% of their free-feeding body weights. They were then trained to press the levers, and were exposed to a discrete-trials continuous reinforcement schedule, in which the two levers were presented in a random sequence, for three sessions. Thereafter, the rats underwent 50-minute training sessions under the discrete-trials psychophysical procedure, as described in Experiment 1 (see Section 2.2.3).

5.2.4. Surgery

Surgical preparation took place after >90 sessions of preliminary training under the discrete-trials psychophysical procedure. The rats were anaesthetized with 4% halothane in oxygen, and placed in a stereotaxic apparatus, with the incisor bar fixed 3.3 mm below the inter-aural line; anaesthesia was maintained with 2% halothane in oxygen during the surgery. Bilateral 22-gauge guide cannulae (Plastics One Inc., Roanoke, VA, USA) were introduced into the brain via 1-mm holes drilled in the skull, and their tips were positioned at the dorsal margin of the corpus striatum, according to the following stereotaxic coordinates: AP +1.0 mm, L \pm 2.5 mm, DV -4.0 mm, measured from bregma (Paxinos and Watson 1998). Three stainless steel anchor screws were placed in the skull, the cannula assembly was fixed to the skull with dental cement, and the wound was closed around the cannula assembly. Stylets were introduced into the guide cannulae, and the

assembly was covered by a plastic screw cap. The rats were returned to the daily training routine on the day following surgery.

5.2.5. **Drug treatment**

Two weeks after surgery, the rats were acclimatized to manual restraint and the intracerebral injection procedure over a number of sessions before starting the drug treatment regimen. Intracerebral injections were given via bilateral 28-gauge injection canulae which protruded 1 mm below the tips of the guide cannulae. Sterile drug solutions or vehicle (see below) were infused at a rate of $0.2 \mu\text{l min}^{-1}$ via polythene tubes connected to 100- μl Hamilton syringes driven by a dual syringe pump (Linton Instrumentation, Diss, UK). The volume injected was always 0.5 μl (total injection time, 2.5 minutes). The injection cannulae remained in place for one minute after the completion of the injection to allow for diffusion within the tissue. The cannulae were then removed and the stylets replaced, and the rats were returned to their home cages for 2-3 minutes before being placed in the operant conditioning chambers. The experimental session began six minutes after completion of the injection. Intracerebral injections were given twice a week, with at least 72 hours between successive injections. A maximum of 12 injections were given to each rat.

The rats were divided into three groups. Group 1 ($n=10$) received the following three treatments, each treatment being given four times: (i) vehicle (intracerebral [i.c.]), (ii) DOI (1 μg , i.c.), (iii) DOI (3 μg , i.c.). Group 2 ($n=10$) received the following three treatments, each treatment being given four times: (i) vehicle (i.c.), (ii) MDL-100907 (0.1 μg , i.c.), (iii) MDL-100907 (0.3 μg , i.c.). Group 3 ($n=9$) received the following four treatments, each treatment being given

three times: (i) vehicle (s.c.) + vehicle (i.c.), (ii) DOI (0.25 mg kg⁻¹, s.c.) + vehicle (i.c.), (iii) DOI (0.25 mg kg⁻¹, s.c.) + MDL-100907 (0.3 µg, i.c.), (iv) DOI (0.25 mg kg⁻¹, s.c.) + RS-102221 (0.15 µg, i.c.).

The doses for intracerebral injection were chosen on the basis of previous studies in which these compounds were injected intracerebrally (for references, see Discussion). The protocol for subcutaneous injections was the same as in Experiment 1, (see Section 2.2.4.).

DOI was dissolved in sterile water, MDL-100907 was dissolved in glacial acetic acid, and RS-102221 [8-(5-(2,4-dimethoxy-5-(trifluoromethylphenylsulphonamido)phenyl-5-oxopentyl)1,3,8-triazaspiro(4.5)decane-2,4-dione HCl] was dissolved in 0.9% sodium chloride solution. Each drug solution was diluted to volume with phosphate buffered saline (pH 7.0). Doses of the drug refer to the weights of the salts.

5.2.6. Histology

At the end of the experiment, the rats were killed by CO₂ and their brains were removed and fixed in 10% formol saline for one week. The brains were sectioned using a freezing microtome. Coronal sections (60 µm) taken through the striatum were mounted on gelatine-coated slides. The selected sections were dried in formaldehyde vapour and placed through the following series of solutions: 95% ethanol (15 min), 70% ethanol (1 min), 50% ethanol (1 min), distilled water (2 min), 0.25% cresyl violet (2 min), distilled water (1 min), 50% ethanol (1 min), 70% ethanol (2 min), 95% ethanol (2 min), 100% ethanol (1 min), xylene (5 min). Slides were mounted with DPX and coverslipped. An investigator who was blind to the behavioural results performed the microscopic examination. Drawings of

the locations of the cannula tips were superimposed on the appropriate pages of the stereotaxic atlas of Paxinos and Watson (1998).

5.2.7. **Data analysis**

The data from the three groups (Group 1: intra-striatally administered DOI; Group 2: intra-striatally administered MDL-100907; Group 3: systemically administered DOI combined with intra-striatally administered MDL-100907 and RS102221) were analysed separately. The methods of analysis were similar to those used in Experiments 1 and 3 (see Section 2.2.5).

For each treatment, the percentages of responses emitted on lever B (%B) at each time-point were analysed by two-factor analyses of variance (treatment \times time) with repeated measures on both factors. In the event of a significant main effect of treatment or a significant treatment \times time interaction, analyses of the simple main effect at each time-point were carried out, followed by comparisons between each active treatment with the control (vehicle-alone) condition using Dunnett's test. In the case of data from the drug interaction study (Group 3), multiple comparisons were made between treatment with DOI + vehicle and the combined DOI + antagonist treatments, using Neuman-Keul's test. Quantitative analysis of the psychometric functions was identical to experiment 1 (see Section 2.2.5.)

5.3. **RESULTS**

Under each treatment condition, proportional choice of lever B (%B) increased progressively as a function of stimulus duration, t . Under the vehicle-alone

condition and all active treatment conditions, the number of ‘missed’ trials (i.e. trials in which no response was emitted on either lever A or lever B) was <0.5%.

5.3.1. Intra-striatal administration of DOI (Group 1)

The effect of DOI (1, 3 µg) on proportional choice of lever B (%B) is shown in Fig. 5.1. Analysis of variance of these data revealed that the main effect of the treatment was not statistically significant [$F(2,16) = 1.0, P > 0.1$]. The main effect of time was statistically significant [$F(9,72) = 267.7, P < 0.001$]. There was no significant treatment \times time interaction [$F(18,144) = 1.0, P > 0.1$].

Logistic functions were fitted to the data from each rat under each treatment condition; the group mean values of the parameters of these functions (\pm SEM) are shown in Table 5.1. There was no significant effect of treatment on T_{50} , ϵ or the Weber fraction [$F < 1$ in each case].

5.3.2. Intra-striatal administration of MDL-100907 (Group 2)

The effect of MDL-100907 (1, 3 µg) on proportional choice of lever B (%B) is shown in Fig. 5.2. Analysis of variance of these data revealed that the main effect of the treatment was not statistically significant [$F < 1$]. The main effect of time was statistically significant [$F(9,81) = 207.6, P < 0.001$]. There was no significant treatment \times time interaction [$F(18,162) = 1.5, P > 0.05$].

Logistic functions were fitted to the data from each rat under each treatment condition; the group mean values of the parameters of these functions (\pm SEM) are shown in Table 5.2. There was no significant effect of treatment on T_{50} , ϵ [$F < 1$ in each case], or the Weber fraction [$F(2,28) = 1.7, P > 0.05$].

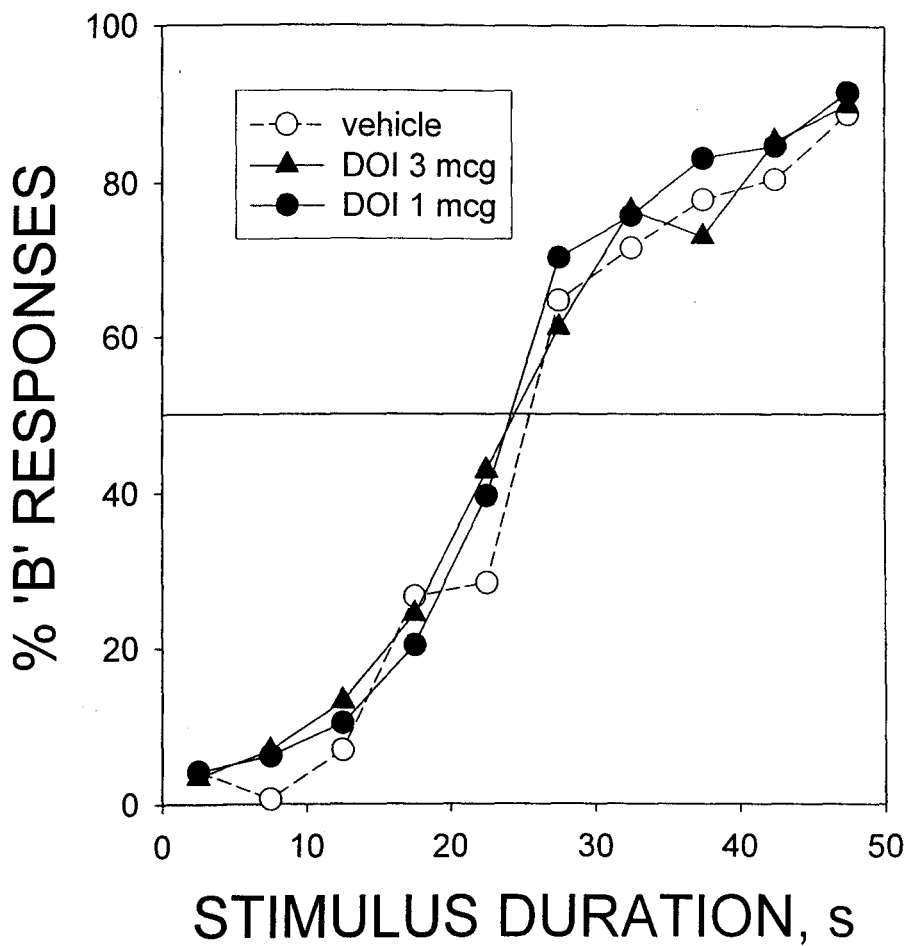


Figure 5.1. Effect of intra-striatally administered DOI (1, 3 μ g) on the relationship between proportional choice of lever B (%B) and stimulus duration (t , seconds) in the discrete-trials psychophysical procedure. Points indicate group mean data under each treatment condition (see inset).

Table 5.1. Effects of the intra-striatally administered DOI on measures of performance on the discrete-trials psychophysical procedure: group mean values (\pm SEM)

Treatment	Parameters of logistic psychometric function			
	T_{50}, s	slope, ϵ	p^2	Weber fraction
Vehicle	26.1 \pm 1.1	-4.6 \pm 1.3	0.93 \pm 0.01	0.32 \pm 0.04
DOI 1 μ g	24.4 \pm 0.9	-4.4 \pm 0.6	0.95 \pm 0.01	0.29 \pm 0.04
DOI 3 μ g	24.5 \pm 1.1	-3.6 \pm 0.6	0.92 \pm 0.02	0.37 \pm 0.06

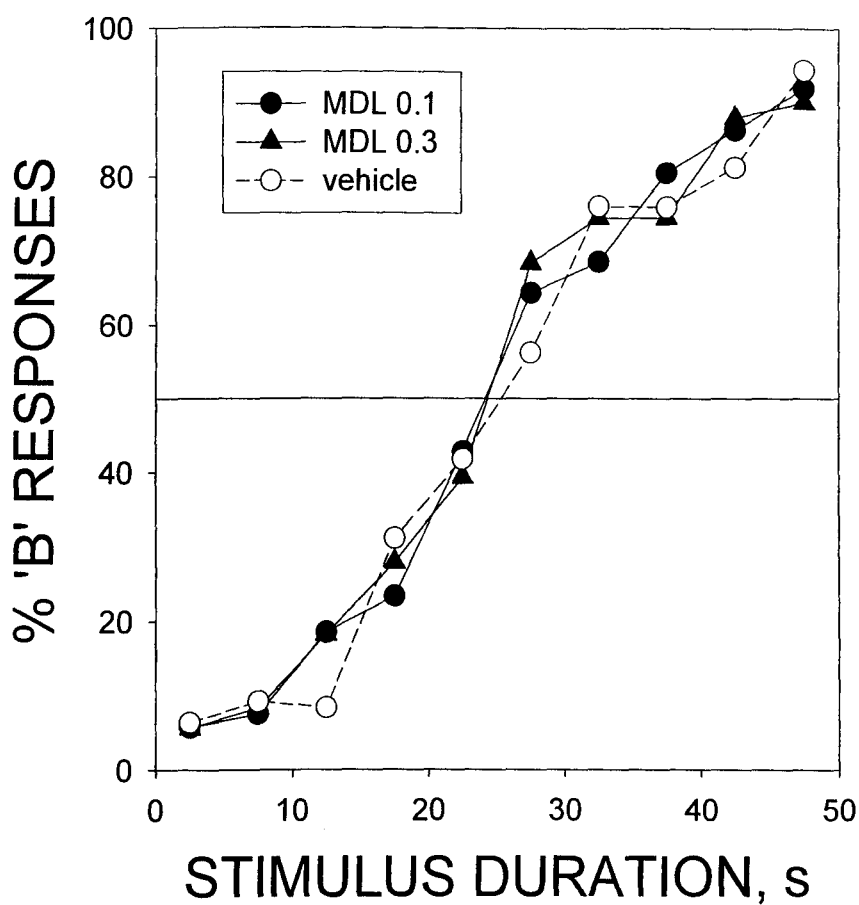


Figure 5.2. Effect of intra-striatally administered MDL-100907 (0.1, 0.3 μ g) on the relationship between proportional choice of lever B (%B) and stimulus duration (t , seconds) in the discrete-trials psychophysical procedure. Points indicate group mean data under each treatment condition (see inset).

Table 5.2. Effects of intra-striatally administered MDL100907 on measures of performance on the discrete-trials psychophysical procedure: group mean values (\pm SEM)

Treatment	Parameters of logistic psychometric function			
	T_{50}, s	slope, ϵ	p^2	Weber fraction
Vehicle	24.8 \pm 0.9	-3.30 \pm 0.20	0.74 \pm 0.01	0.35 \pm 0.02
MDL 100907 1 μ g	24.1 \pm 1.2	-3.41 \pm 0.33	0.92 \pm 0.10	0.36 \pm 0.40
MDL 100907 3 μ g	23.7 \pm 1.1	-3.18 \pm 0.32	0.92 \pm 0.00	0.43 \pm 0.04

5.3.3. Interaction between systemically administered DOI with intrastrially administered MDL-100907 and RS-102221 (Group 3)

The %B data are shown in Fig. 5.3. Analysis of variance of these data revealed a significant main effect of time [$F(9,81) = 64.9, P < 0.001$]. There was no significant main effect of treatment [$F < 1$]. However, there was a significant treatment \times time interaction [$F(27, 243) = 2.7, P < 0.001$]. Analysis of the simple main effects revealed significant treatment effects 37.5 s [$F(3,27) = 5.8, P < 0.005$] and 47.5 s [$F(3,27) = 4.3, P < 0.05$] after trial onset. Multiple comparisons showed that DOI produced a significant decrease in %B at both these time points; in neither case was this effect significantly reversed by either MDL-100907 or RS-102221.

Logistic functions were fitted to the data from each rat under each treatment condition; the group mean values of the parameters of these functions (\pm SEM) are shown in Table 5.3. There was no significant effect of treatment on T_{50} [$F(3,21) = 1.3, P > 0.1$]. The effect of treatment on ϵ was statistically significant [$F(3,21) = 5.6, P < 0.005$]. *Post hoc* tests showed that the value of this parameter was increased by systemically administered DOI; however DOI's effect was not significantly reversed by either MDL-100907 or RS-102221. The effect of treatment on the Weber fraction fell short of statistical significance [$F(3,21) = 2.6, 0.1 > P > 0.05$].

5.3.4. Histology

Figure 5.4 shows the cannula placements for all the rats. In each rat, the tracks of the internal cannulae terminated in the dorsal striatum in both hemispheres.

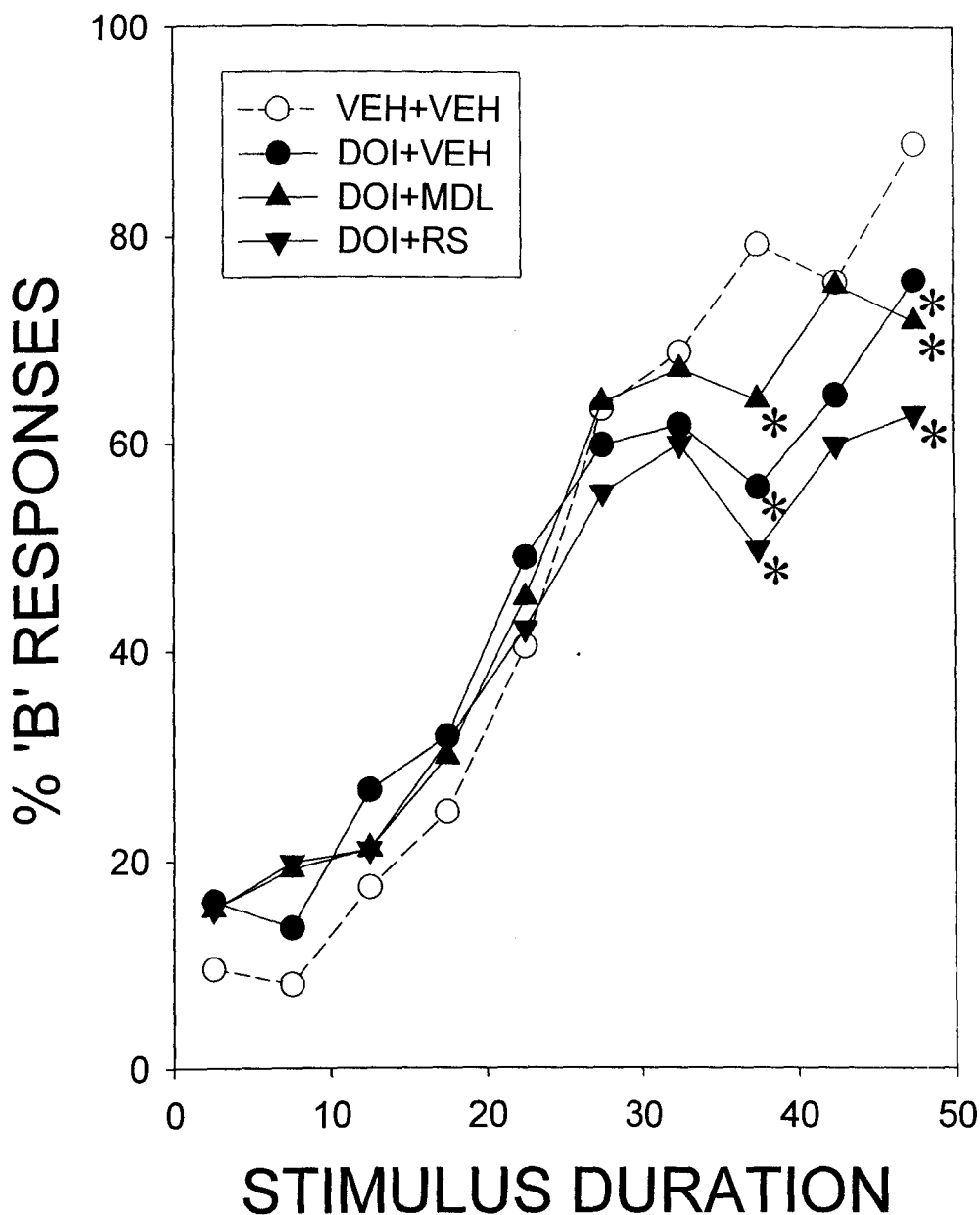


Figure 5.3. Interaction between systemically administered DOI and intrastrially administered MDL-100907 and RS-102221 on the relationship between proportional choice of lever B (%B) and stimulus duration in the discrete-trials psychophysical procedure. DOI significantly reduced %B at the 37.5 and 47.5 s time points (* $P < 0.05$). In neither case was this effect significantly altered by either MDL-100907 or RS-102221 (see text for details).

Table 5.3. Interaction between systemically administered DOI and intra-striatally administered MDL-100907 and RS102221 on measures of performance on the discrete-trials psychophysical procedure: group mean values (\pm SEM)

Treatment	Parameters of logistic psychometric function			
	T_{50} , s	slope, ϵ	p^2	Weber fraction
Vehicle + Vehicle	23.0 \pm 1.7	-3.3 \pm 0.5	0.88 \pm 0.03	0.42 \pm 0.08
DOI + Vehicle	26.8 \pm 2.1	-1.9 \pm 0.2*	0.83 \pm 0.04	0.69 \pm 0.10
DOI + MDL-100907	27.6 \pm 4.8	-2.2 \pm 0.3*	0.78 \pm 0.04	0.59 \pm 0.08
DOI + RS-102221	31.0 \pm 4.0	-1.6 \pm 0.4*	0.64 \pm 0.10	0.99 \pm 0.33

* significantly different from control (Vehicle + Vehicle) condition ($P<0.05$); see text for details

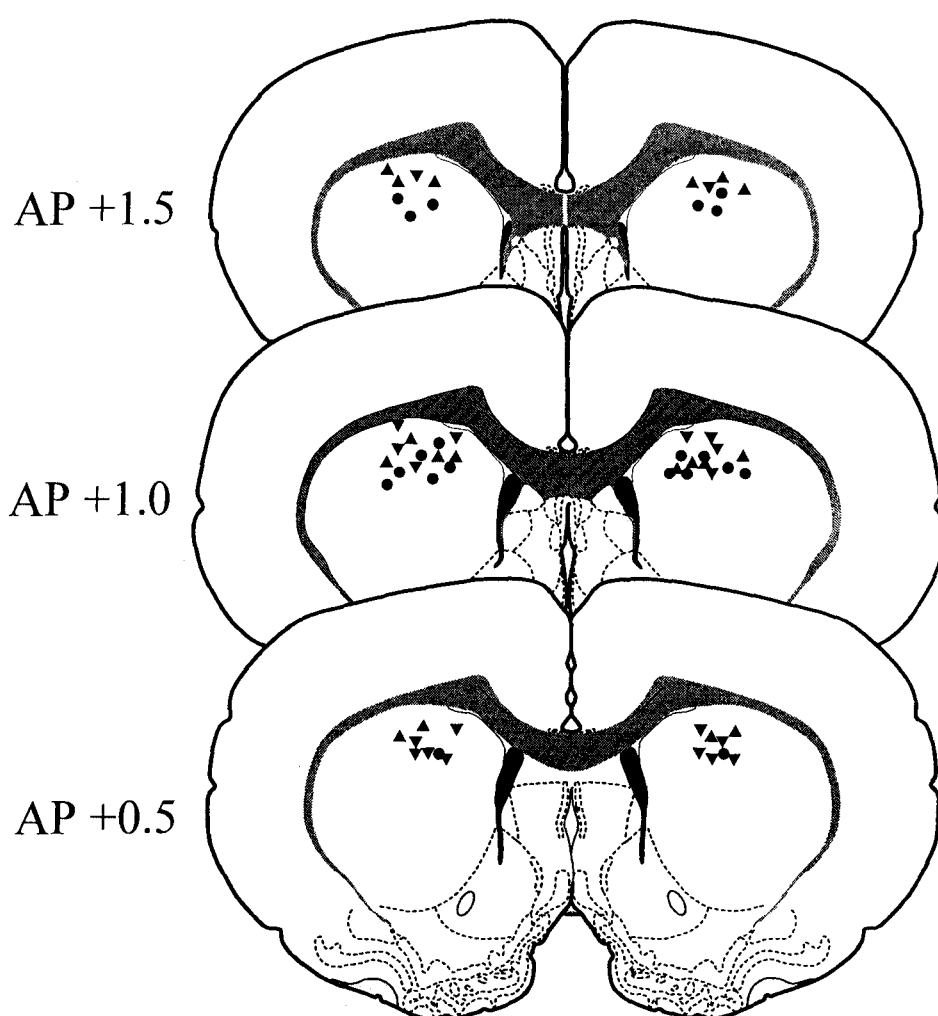


Figure 5.4. Diagram of cannula placements within the dorsal striatum. Points indicate the approximate positions of the tips of the injection cannulae derived from the histological slides (see text for details). The three sections are taken from Paxinos and Watson's atlas at the AP locations indicated. *Circles*: brains of rats from Group 1; *triangles*: brains of rats from Group 2; *inverted triangles*: brains or rats from Group 3.

5.4. DISCUSSION

Temporal discrimination performance in the discrete-trials psychophysical procedure used in this experiment was similar to that reported previously: proportional choice of lever B increased as a sigmoid function of stimulus duration, this being well described by a two-parameter logistic equation (Body et al. 2002a; see also Chapters 2 and 4). The fits of the logistic functions were somewhat poorer in this experiment than in the previous experiments employing the discrete-trials psychophysical procedure (Experiments 1 and 3). This probably reflects the fact that in the present experiment the data were derived from only three or four sessions under each treatment condition, as opposed to five sessions in the other experiments. The smaller number of treatment sessions in the present experiment was due to the limited number of intracerebral injections that it was considered appropriate to give to each rat. The giving of twelve injections seems to be justified, in that the histological examination of the brains did not indicate any significant structural damage to the striatum. Further experiments may be needed to ascertain whether this number could be increased without incurring unacceptable tissue damage.

Systemically administered DOI (Group 3) produced some disruption of temporal discrimination, although the effect was less marked than in Experiment 3. In the present experiment, the slope of the psychometric function was significantly flattened by DOI; however, in contrast to Experiment 3, T_{50} and the Weber fraction were not significantly altered. It is possible that this reflects the smaller number of animals and the smaller number of injections given to each animal in the present experiment (see above).

There is a substantial body of evidence indicating that the dorsal striatum

plays a pivotal role in the regulation of interval timing behaviour (Harrington et al. 1998; Meck and Benson 2001; Matell et al. 2003; Nenadic et al. 2003; Hinton and Meck 2004; Matell and Meck 2004; Pastor et al. 2004; Lustig et al. 2005; Meck 2005). 5-HT_{2A} receptors exist in considerable numbers in the striatum (see Barnes and Sharp 1999; Hoyer et al. 2002), where they may contribute to the regulation of the activity of the direct striatal output pathway (Bishop et al. 2004). Therefore it was decided, in the present experiment, to examine whether the 5-HT_{2A} receptor population responsible for DOI's effect on temporal discrimination might be located in the dorsal striatum. However, DOI had no significant effect on temporal discrimination when it was injected directly into the dorsal striatum (Group 1). Furthermore, MDL-100907, administered directly into the dorsal striatum, had no effect on temporal discrimination (Group 2), and was not able to attenuate the effects of systemically administered DOI on the slope of the psychometric function (Group 3). Thus the present results suggest that the population of 5-HT_{2A} receptors that mediates DOI's effects on temporal discrimination probably does not reside in the dorsal striatum.

The failure of intra-striatally injected RS-102221 to block the effect of DOI argues against a significant role of striatal 5-HT_{2C} receptors in DOI's effect, because RS-102221 has a considerably higher affinity for 5-HT_{2C} receptors than for other 5-HT₂ receptor subtypes (Bonhaus et al. 1997). It has yet to be established whether systemic administration of 5-HT_{2C} receptor antagonists can alter the effect of DOI on timing behaviour.

The possibility cannot be totally excluded that the lack of effect of the intracerebrally administered drugs in these experiments was due to the use of inadequate doses. However, intracerebral injection of DOI in doses comparable to those used in the present experiments has been found to be effective in other

behavioural tests (Sipes and Geyer 1997; Bishop et al. 2004). Moreover, doses of MDL-100907 and RS-102221 similar to those used in the present experiment have been found to attenuate cocaine-induced behaviour when injected into the ventral tegmental area and nucleus accumbens, respectively (McMahon et al. 2001; Filip and Cunningham 2002). Therefore, it seems reasonable to interpret the inability of DOI and the two antagonists to affect temporal discrimination following direct injection into the dorsal striatum as indicating that the relevant receptor population is not located in this structure.

CHAPTER 6

Experiments 5 and 6:

EFFECTS OF SYSTEMICALLY AND INTRA- STRIATALLY ADMINISTERED DOI ON TEMPORAL DIFFERENTIATION

Experiment 5: Effects of systemically administered DOI and MDL-100907

Experiment 6: Effects of intra-striatally administered DOI, MDL-100907 and RS-102221

6.1. INTRODUCTION

The experiments described in the previous chapter investigated the hypothesis that the disruptive effects of 5-HT₂ receptor agonists on temporal discrimination might be mediated by a 5-HT_{2A} receptor population located in the dorsal striatum, an area that has been implicated in the control of interval timing behaviour. The results did not support the hypothesis.

The experiments described in this chapter examined the same hypothesis in the case of *temporal differentiation*. There is good evidence that 5-HT_{2A} receptor stimulation can alter temporal differentiation in the free-operant psychophysical procedure. The results of Experiment 2 (see Chapter 3) showed that the non-selective 5-HT receptor agonist quipazine produced a leftward displacement of the psychometric function derived from this schedule, reflected in a reduction of the indifference point T_{50} . The reversal of this effect by ketanserin strongly suggests that the quipazine's effect was mediated by 5-HT_{2A} receptors. This result is consistent with previous findings by Body et al. (2003, 2004), showing that DOI reduced T_{50} in the free-operant psychophysical procedure, and that this effect could be reduced by ketanserin.

One aim of the experiments described in this chapter was to extend these observations with ketanserin to the highly selective 5-HT_{2A} receptor antagonist MDL-100907. The other aim was to examine whether the effect of systemically administered DOI would be reproduced when the agonist was injected directly into the striatum. In addition it was examined whether intra-striatal injection of MDL-100907 and the highly selective 5-HT_{2C} receptor antagonist RS-102221 (Bonhaus et al. 1997) could block the effect of DOI on temporal differentiation.

6.2. **Experiment 5: EFFECTS OF SYSTEMICALLY ADMINISTERED DOI AND MDL-100907**

6.2.1. **Methods**

6.2.1.1. *Subjects*

Twenty female Wistar rats aged approximately 4 months and weighing 250-290 g at the start of the experiment were housed individually under the same conditions as in Experiment 1.

6.2.1.2. *Apparatus*

The rats were trained in operant conditioning chambers (CeNeS Ltd, Cambridge, UK) identical to those used in Experiment 4 (see Section 5.2.1).

6.2.1.3. *Behavioural training*

At the start of the experiment, the food deprivation regimen commenced and the rats were gradually reduced to 80% of their free-feeding body weights. They were then trained to press the levers, and were exposed to a discrete-trials continuous reinforcement schedule, in which the two levers were presented in a random sequence, for three sessions. Thereafter, the rats underwent 50-minute training sessions under the free-operant psychophysical procedure as described in Experiment 2 (see Chapter 3 for details).

6.2.1.4. *Drug treatment*

The drug treatment regimen started after 80 sessions of preliminary training under the free-operant psychophysical procedure. DOI was injected subcutaneously using a 26-gauge needle, at a volume of 1.0 ml kg^{-1} , 15 minutes before the start of the experimental session. MDL-100907 was injected intraperitoneally using a 25-gauge needle, at a volume of 2.5 ml kg^{-1} , 25 minutes before the start of the session. Drugs were administered on Tuesdays and Fridays, vehicle injections were given on Mondays and Thursdays, and no injections were given on Wednesdays, Saturdays or Sundays. The order of treatments was balanced within and between animals according to a Latin square. Control injections used the vehicle appropriate for that drug (see below). Each treatment was administered five times in order to accrue a sufficient number of probe trials to obtain reliable estimates of the timing indices for individual rats (Chiang et al. 2000 a, b). Each rat received DOI 0.25 mg kg^{-1} , MDL-100907 0.5 mg kg^{-1} , and a combined treatment with DOI 0.25 mg kg^{-1} + MDL-100907 0.5 mg kg^{-1} . Doses of the drugs refer to the weights of the salts.

DOI was dissolved in 0.9% sodium chloride solution. MDL-100907 was dissolved in glacial acetic acid and sterile water, buffered to pH 5.5 and diluted to volume with 0.9% sodium chloride solution.

6.2.1.5. *Data analysis*

Only the data collected from the probe trials were used in the analysis. The methods for data analysis were similar to those used in Experiment 2 (see Section 3.2.5.).

Relative response rates. Relative response rate on lever B (%B), defined as the response rate on lever B divided by the combined response rate on both levers, was analysed by a two-factor analysis of variance (treatment \times time-bin) with repeated measures on both factors.

Psychometric functions. A two-parameter logistic function was fitted to the relative response rate data from each rat under each treatment condition: $\%B = 100 / (1 + [t/T_{50}]^\epsilon)$, where t is time from trial onset, T_{50} (the indifference point) is a parameter expressing the time at which $\%B = 50\%$, and ϵ is the slope of the function; these parameters were used to derive the Weber fraction, as described previously (see Section 3.2.5). The values of T_{50} , ϵ , and the Weber fraction were analysed by one-factor analyses of variance (treatments) with repeated measures. In the case of a significant effect of treatment, comparisons were made between each active treatment and the control (vehicle alone) condition using Dunnett's test. In the case of data from agonist-antagonist interaction, multiple comparisons were made between treatment with DOI alone and the combined DOI + MDL-100907 treatment, using the Newman-Keuls test (significance criterion, $P < 0.05$).

Overall response rates. Overall response rate was analysed using a one-factor analysis of variance (treatment), with repeated measures, followed by *post-hoc* analyses as described above.

Switching. The probability of a switch occurring in each 5-s epoch of the probe trials was calculated for each rat. Logistic functions (see above) were fitted to the cumulative probability distributions, and the inflection point, S_{50} , was derived (see Section 3.2.5.) The values of S_{50} were subjected to one-factor analyses of variance, as described above.

6.2.2. Results

Psychometric functions

In each treatment condition, response rate on lever A declined and response rate on lever B increased as a function of time from trial onset, the proportion of responding allocated to lever B (%B) increasing progressively during the course of the trial (Figure 6.1). Analysis of variance revealed significant main effects of time-bin [$F(9,171)=453.6$, $P<0.001$] and treatment [$F(3,57)=5.3$, $P<0.01$], and a significant treatment \times time-bin interaction [$F(27,513)=5.2$, $P<0.001$].

Logistic functions were fitted to the %B data from each treatment condition; the group mean values of the parameters of these functions (\pm SEM) are shown in Table 6.1.

Indifference point, T_{50} . Analysis of variance showed a significant effect of treatment [$F(3,57)=7.6$, $P<0.001$]. Multiple comparisons showed that DOI significantly reduced T_{50} . The effect of DOI was significantly reversed by co-administration of MDL-100907; there was no significant difference between the values of T_{50} derived from the vehicle and DOI + MDL-100907 conditions.

Slope. There was no significant effect of treatment ($F<1$).

Goodness of fit, p^2 . The mean values of p^2 were >0.97 under each treatment condition.

Weber fraction. There was no significant effect of treatment [$F(3,57)=1.3$, $P>0.1$].

Overall response rates. The group mean overall response rates (\pm SEM) under each treatment condition are shown in Table 6.1. There was a significant effect of treatment [$F(3,57)=30.4$, $P<0.001$]. Multiple comparisons showed that

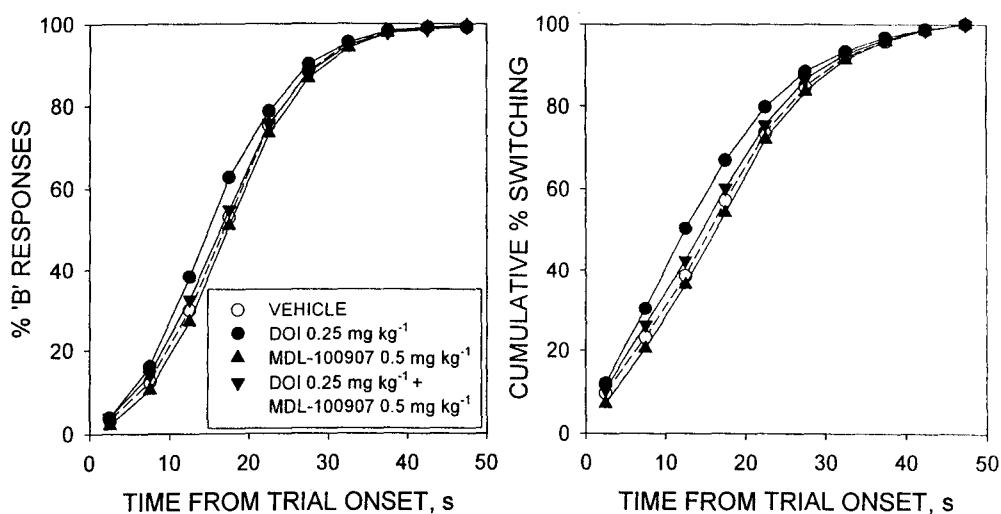


Figure 6.1. A. Effect of systemic treatment with DOI, MDL-100907 and combined treatment with DOI + MDL-100907 on performance on the free-operant psychophysical procedure. *Ordinate*: percent responding on lever B (%B); *abscissa*: time from trial onset (s). Points indicate group mean data under each treatment condition (see inset). **B.** Effect on the treatments on probability of switching from lever A to lever B. *Ordinate*: cumulative probability of switching; *abscissa*: time from trial onset (s).

Table 6.1. Experiment 5: Effects of the treatments timing performance on the free-operant psychophysical procedure

Treatment	Mean \pm SEM				
	T_{50} (s)	ε	Weber fraction	Response rate (responses min ⁻¹)	S_{50} (s)
Vehicle	13.77 \pm 0.20	-3.79 \pm 0.20	0.31 \pm 0.02	55.97 \pm 1.20	12.05 \pm 0.74
DOI 0.25 mg kg ⁻¹	11.75 \pm 0.84*	-4.53 \pm 0.59	0.31 \pm 0.03	36.66 \pm 2.13*	9.62 \pm 0.74*
MDL-100907 0.5 mg kg ⁻¹	14.62 \pm 0.73	-4.48 \pm 0.25	0.26 \pm 0.01	61.29 \pm 1.34	12.82 \pm 0.76
DOI 0.25 mg kg ⁻¹ + MDL-100907 0.5 mg kg ⁻¹	13.97 \pm 0.83 [#]	-4.18 \pm 0.30	0.29 \pm 0.02	53.01 \pm 1.19 [#]	11.25 \pm 0.82 [#]

DOI significantly reduced the overall response rate, and that this effect was significantly attenuated by MDL-100907.

Switching. DOI displaced the switching probability function to the left, and this effect was reversed by MDL-100907 (Figure 6.1). There was a significant effect of treatment on S_{50} [$F(3,57)=6.7$, $P<0.01$]. Multiple comparisons showed that DOI reduced S_{50} and that this effect was attenuated by co-administration of MDL-100907; there was no significant difference between the values of S_{50} derived from the vehicle and DOI + MDL-100907 conditions (Table 6.1).

6.3. Experiment 6: EFFECTS OF INTRA-STRIATALLY ADMINISTERED DOI, MDL-100907 AND RS-102221

6.3.1. Methods

6.3.1.1. Subjects

Eighteen female Wistar rats aged approximately 4 months and weighing 250-290 g at the start of the experiment were housed individually under the same conditions as in the previous experiment.

6.3.1.2. Apparatus

The same apparatus was used as in the previous experiment.

6.3.1.3. *Behavioural training*

The rats were trained under the same schedule as that used in the previous experiment.

6.3.1.4. *Surgery*

Surgical preparation was identical to that used in Experiment 4 (see Section 5.2.4).

6.3.1.5. *Drug treatment*

Two weeks after surgery the rats were divided into two groups. Group 1 ($n=9$) received the following three treatments, each treatment being given four times: (i) vehicle (intracerebral [i.c.]), (ii) DOI (1 μg , i.c.), (iii) DOI (3 μg , i.c.). Group 2 ($n=9$) received the following four treatments, each treatment being given three times: (i) vehicle (s.c.) + vehicle (i.c.), (ii) DOI (0.25 mg kg^{-1} , s.c.) + vehicle (i.c.), (iii) DOI (0.25 mg kg^{-1} , s.c.) + MDL-100907 (0.3 μg , i.c.), (iv) DOI (0.25 mg kg^{-1} , s.c.) + RS-102221 (0.15 μg , i.c.). The doses for intracerebral injection were chosen on the basis of previous studies in which these compounds were injected intracerebrally (for references, see Discussion). The protocol for subcutaneous injections was the same as in Experiment 5, and for intra-cerebral injections was the same as in Experiment 4.

DOI was dissolved in sterile water, MDL-100907 was dissolved in glacial acetic acid, and RS-102221 [8-(5-(2,4-dimethoxy-5-(trifluoromethylphenyl)-

sulphonamido)phenyl-5-oxopentyl)1,3,8-triazaspiro(4.5)decane-2,4-dione HCl] was dissolved in 0.9% sodium chloride solution. Each drug solution was diluted to volume with phosphate buffered saline (pH 7.0). Doses of the drug refer to the weights of the salts.

6.3.1.6. *Histology*

Histological analysis was the same as experiment 4 (see Section 5.2.6).

6.3.1.7. *Data analysis*

The data from the two groups (Group 1: DOI, i.c.; Group 2: DOI, s.c. + MDL-100907, i.c. and RS-102221, i.c.) were analysed separately. The same methods of analysis were used as in Experiment 5 (see Section 6.2.1.7).

6.3.2. **Results**

6.3.2.1. *Intra-striatal administration of DOI (Group 1)*

Psychometric functions. Under all treatment conditions, response rate on lever A declined and response rate on lever B increased as a function of time from trial onset, the proportion of responding devoted to lever B (%B) increasing progressively during the course of the trial (Figure 6.2). Analysis of variance (treatment \times time-bin) revealed significant main effects of time-bin [$F(9,72)=410.8, P<0.001$] and treatment [$F(2,16)=4.9, P<0.05$], and a significant treatment \times time-bin interaction [$F(18,144)=3.2, P<0.001$]. The parameters of the fitted curves are shown in Table 6.2.

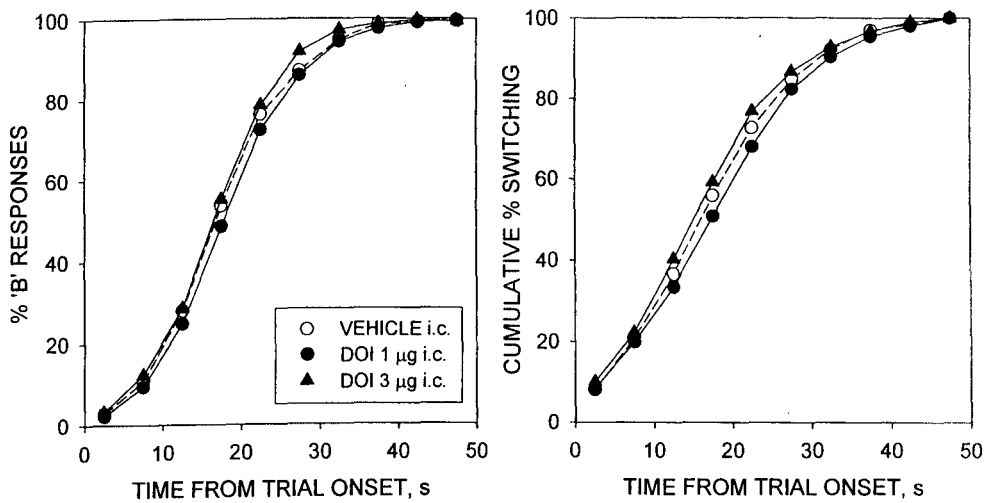


Figure 6.2. Effects of intra-striatal administration of DOI (1 and 3 µg) on performance on the free-operant psychophysical procedure. Conventions as in Figure 6.1.

Table 6.2. Experiment 6: effects of the treatments on timing performance on the free-operant psychophysical procedure

Treatment	Mean \pm SEM				
	T_{50} (s)	ϵ	Weber fraction	Response rate (responses min ⁻¹)	S_{50} (s)
Vehicle (i.c.)	16.31 \pm 1.03	-4.03 \pm 0.22	0.28 \pm 0.02	50.55 \pm 3.02	14.92 \pm 0.87
DOI 1 μ g (i.c.)	17.56 \pm 1.00	-4.17 \pm 0.31	0.28 \pm 0.02	50.43 \pm 3.03	15.93 \pm 0.94
DOI 3 μ g (i.c.)	15.91 \pm 0.78	-4.09 \pm 0.27	0.28 \pm 0.02	51.65 \pm 3.49	14.02 \pm 0.58
Vehicle (s.c.) + vehicle (i.c.)	17.52 \pm 1.42	-4.13 \pm 0.24	0.28 \pm 0.02	44.04 \pm 3.63	13.61 \pm 1.37
DOI 0.25 mg kg ⁻¹ (s.c.) + vehicle (i.c.)	14.82 \pm 1.54*	-3.17 \pm 0.32*	0.38 \pm 0.03*	30.44 \pm 2.81*	10.43 \pm 1.12*
DOI 0.25 mg kg ⁻¹ (s.c.) + MDL-100907 0.3 μ g (i.c.)	15.50 \pm 1.69*	-3.29 \pm 0.31*	0.37 \pm 0.04*	34.38 \pm 3.23*	10.64 \pm 1.49*
DOI 0.25 mg kg ⁻¹ (s.c.) + RS-102221 0.15 μ g (i.c.)	14.97 \pm 1.28*	-3.30 \pm 0.26*	0.36 \pm 0.03*	32.96 \pm 2.06*	10.93 \pm 1.29*

* Significantly different from vehicle ($P < 0.05$)

Indifference point, T_{50} . There was a significant effect of treatment [$F(2,16)=4.4$, $P<0.05$]. However, multiple comparisons with the vehicle alone-condition revealed that neither dose of DOI produced a significant effect on T_{50} .

Slope. There was no significant effect of treatment ($F<1$).

Goodness of fit, p^2 . The mean values of p^2 were >0.99 under each treatment condition.

Weber fraction. There was no significant effect of treatment ($F<1$).

Overall response rate. There was no significant effect of treatment ($F<1$).

Switching, S_{50} . Analysis of variance revealed a significant main effect of treatment [$F(2,16)=5.1$, $P<0.05$]. However, multiple comparisons with the vehicle-alone treatment revealed that neither dose of DOI produced a significant effect on S_{50} .

6.3.2.2. *Intra-striatal administration of MDL-100907 and RS-102221: interaction with systemically administered DOI (Group 2)*

Psychometric functions. Under all treatment conditions, response rate on lever A declined and response rate on lever B increased as a function of time from trial onset, %B increasing progressively during the course of the trial (Figure 6.3). Analysis of variance of the relative response rate data (treatment \times time-bin) revealed a significant effect of time-bin [$F(9,72)=207.8$, $P<0.001$]. The main effect of treatment was not significant [$F(3,24)=1.9$, $P>0.05$], but there was a significant treatment \times time-bin interaction [$F(27,216)=51.1$, $P<0.001$]. DOI displaced the psychometric function to the left compared to the function derived for the vehicle-alone treatment condition. Neither MDL-100907 nor RS-102221 reversed this effect of DOI. This was confirmed by statistical analysis of the

parameters of the logistic functions derived from the four treatment conditions, shown in Table 6.2.

Indifference point, T_{50} . There was a significant effect of treatment [$F(3,24)=3.5, P<0.05$]. Multiple comparisons showed that DOI reduced T_{50} and that the value of T_{50} obtained in the DOI + vehicle condition did not differ significantly from that obtained in the DOI + MDL-100907 and DOI + RS-102221 conditions, indicating that neither antagonist attenuated DOI's effect on T_{50} .

Slope. There was a significant effect of treatment [$F(3,24)=8.7, P<0.001$]. DOI produced an increase of the value of ε , and hence a flattening of the psychometric curve. Multiple comparisons showed that neither MDL-100907 nor RS-102221 attenuated this effect of DOI.

Goodness of fit, p^2 . The mean values of p^2 were >0.97 under each treatment condition.

Weber fraction. There was a significant effect of treatment [$F(3,24)=6.6, P<0.01$]. Multiple comparisons showed that DOI increased the Weber fraction and that neither MDL-100907 nor RS-102221 reversed this effect of DOI.

Overall response rate. There was a significant effect of treatment [$F(3,24)=15.4, P<0.001$]. Multiple comparisons showed that DOI reduced overall response rate and that neither MDL-100907 nor RS-102221 reversed this effect of DOI.

Switching, S_{50} . DOI reduced the value of S_{50} (Figure 6.3). There was a significant effect of treatment [$F(3,24)=5.8, P<0.01$]. Multiple comparisons revealed a significant effect of DOI; the effect of DOI was not attenuated by either MDL-100907 or RS-102221.

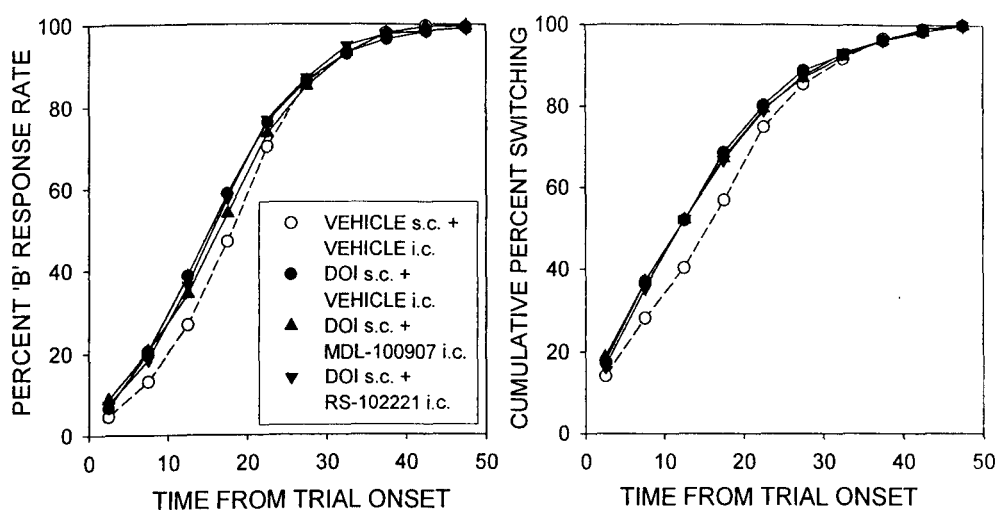


Figure 6.3. Effects of systemic treatment with DOI (0.25 mg kg^{-1}) alone and in combination with intra-striatal administration of MDL-100907 ($0.3 \text{ } \mu\text{g}$) and RS-102221 ($0.15 \text{ } \mu\text{g}$) on performance on the free-operant psychophysical procedure. Conventions as in Figure 6.1.

Figure 6.4 shows the cannula placements for all the rats. In each rat, the tracks of the internal canulae terminated in the dorsal striatum in both hemispheres.

6.4. **DISCUSSION**

In accordance with previous experiments using Stubbs' free-operant psychophysical procedure (Stubbs 1976; Bizo and White 1994a,b; Killeen et al. 1997; Chiang et al. 1998; Machado and Guilhardi 2000; see also Experiment 2), response rate on lever A declined, while response rate on lever B increased, as a function of time from trial onset. This was reflected in an increasing percentage of total responding on lever B (%B) as the trial progressed, that was well described by a two-parameter logistic function.

As has been observed in previous experiments (Body et al. 2003, 2004), systemically administered DOI (0.25 mg/kg) reduced the value of T_{50} . The effect of DOI was completely abolished by systemic co-administration of MDL-100907 (0.5 mg/kg). DOI is a 5-HT₂ receptor agonist with approximately equal affinity for the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor subtypes (see Barnes and Sharp 1999; Hoyer et al. 2002). Body et al. (2003, 2004) previously found that the effect of DOI on T_{50} could be antagonized by ketanserin, an antagonist with considerably higher affinity for the 5-HT_{2A} receptor than for the 5-HT_{2C} receptor (see Hoyer et al. 2002), and concluded that the effect of DOI was probably mediated by 5-HT_{2A} receptors. This conclusion is greatly strengthened by the present results obtained with MDL-100907, a 5-HT_{2A} receptor antagonist with minimal affinity for the

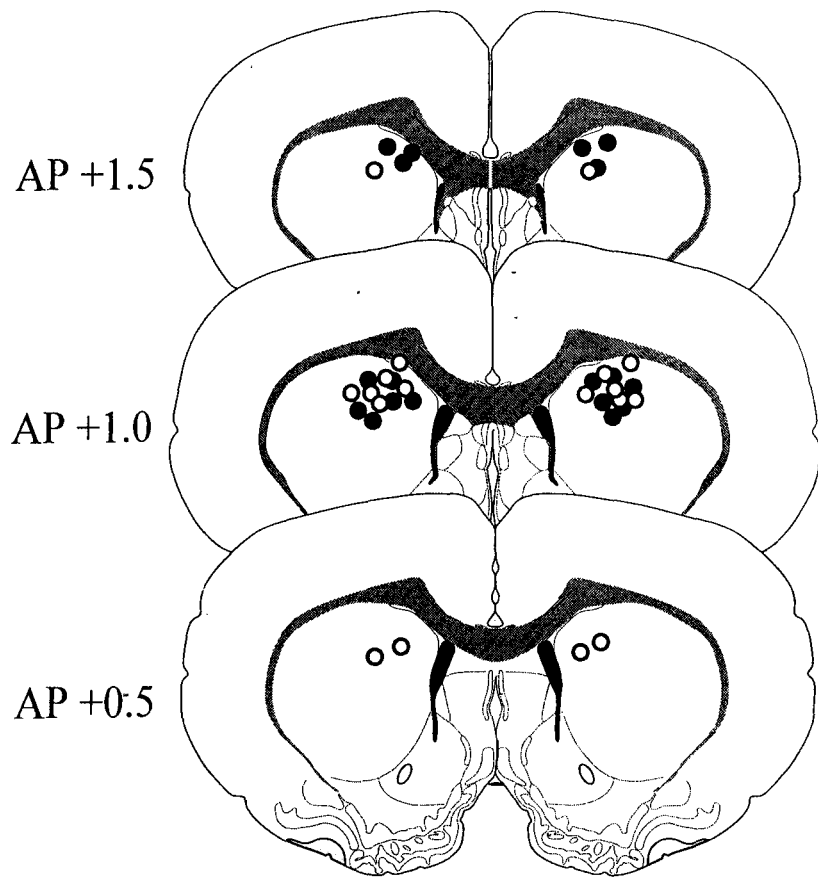


Figure 6.4. Diagram of cannula placements within the dorsal striatum. Points indicate the approximate positions of the tips of the injection cannulae derived from the histological slides (see text for details). The three sections are taken from Paxinos and Watson's atlas at the AP locations indicated. *Open circles*: brains of rats from Group 1; *filled circles*: brains of rats from Group 2.

other 5-HT₂ receptor subtypes (Sorensen et al. 1993; Kehne et al. 1996; Schmidt et al. 1997).

Systemically administered DOI reduced the overall rate of responding on the free operant psychophysical procedure. This effect has been noted before with DOI (Body et al. 2003). The suppression of responding by DOI also appears to be mediated by 5-HT_{2A} receptors, as it was completely reversed by MDL-100907. It is important to note that T_{50} is estimated from relative response rate, and should therefore be impervious to a change in absolute response rates (Chiang et al. 2000a; Odum et al. 2002). It is therefore unlikely that the change in T_{50} was secondary to the change in absolute response rate. Moreover, DOI also produced a decrease in the mean switching time, S_{50} , a measure that is comparable to T_{50} , but which is calculated independently of response rate. The reduction in S_{50} produced by DOI was also reversed by MDL-100907.

As discussed in the previous chapter, there is a substantial body of evidence indicating that the dorsal striatum plays a pivotal role in the regulation of interval timing behaviour (Harrington et al. 1998; Meck and Benson 2001; Matell et al. 2003; Nenadic et al. 2003; Hinton and Meck 2004; Matell and Meck 2004; Pastor et al. 2004; Lustig et al. 2005; Meck 2005). Since 5-HT_{2A} receptors exist in considerable numbers in the striatum (see Barnes and Sharp 1999; Hoyer et al. 2002), it seemed appropriate to examine whether the 5-HT_{2A} receptor population responsible for DOI's effect on temporal differentiation might be located in the dorsal striatum. However, in contrast to its robust effect when administered systemically, DOI had no significant effect on temporal differentiation when it was injected directly into the dorsal striatum. Furthermore, MDL-100907, administered directly into the dorsal striatum, was not able to attenuate the effects of systemically administered DOI on T_{50} , S_{50} or response rate. Thus the present

results suggest that the population of 5-HT_{2A} receptors that mediates DOI's effects on temporal differentiation probably does not reside in the dorsal striatum.

The failure of intra-striatally injected RS-102221 to block the effect of DOI argues against a significant role of striatal 5-HT_{2C} receptors in DOI's effect, because RS-102221 has a considerably higher affinity for 5-HT_{2C} receptors than for other 5-HT₂ receptor subtypes (Bonhaus et al. 1997). As in the case of temporal discrimination (see previous chapter), it remains to be established whether systemic administration of 5-HT_{2C} receptor antagonists can alter the effect of DOI on temporal differentiation.

As discussed in the previous chapter, the possibility cannot be totally excluded that the lack of effect of the intracerebrally administered drugs in these experiments was due to the use of inadequate doses. However, as noted above intracerebral injection of DOI in doses comparable to those used in the present experiments has been found to be effective in other behavioural tests (Sipes and Geyer 1997; Bishop et al. 2004), and doses of MDL-100907 and RS-102221 similar to those used in the present experiments have been found to be behaviourally active when injected into the ventral tegmental area and nucleus accumbens (McMahon et al. 2001; Filip and Cunningham 2002). Therefore, it seems reasonable to interpret the inability of DOI and the two antagonists to affect temporal differentiation following direct injection into the dorsal striatum as indicating that the relevant receptor population is not located in this structure.

Current models of the neural substrate of interval timing generally emphasize the role of the striatum as a component of a cortico-striato-thalamo-cortical loop (Meck and Benson 2001; Ferrandez et al. 2003; Matell et al. 2003; Hinton and Meck 2004; Matell and Meck 2004; Lustig et al. 2005; Meck 2005). 5-HT_{2A} receptors are expressed in more than one component of these loops.

Although they are especially well represented in the dorsal striatum, there are also dense populations in other parts of the basal ganglia and in the cerebral cortex (Pompeiano et al. 1994; Wright et al. 1995; Hamada et al. 1998; Cornea-Hebert et al. 1999; Bubser et al. 2001; Hoyer et al. 2002). In the cortex, in situ hybridization (Burnet et al. 1995; Wright et al. 1995) and single cell recording studies (Marek and Aghajanian 1999) have localized 5-HT_{2A} receptors to glutamatergic corticostriatal projection neurones. In addition, there is evidence that 5-HT_{2A} receptors are present on dopaminergic neurones of the ventral tegmental area and substantia nigra, the nuclei of origin of the forebrain dopaminergic projection (Ikemoto et al. 2000; Nocjar et al. 2002). Whether either or both of these receptor populations is responsible for the effects of 5-HT_{2A} receptor agonists on temporal differentiation is an open question that awaits further investigation.

CHAPTER 7

Experiment 7:

EFFECTS OF 5-HT_{1A} AND 5-HT_{2A} RECEPTOR STIMULATION ON TEMPORAL DIFFERENTIATION PERFORMANCE IN THE FIXED-INTERVAL PEAK PROCEDURE

7.1 INTRODUCTION

As reviewed in Chapter 1, performance on several types of interval timing schedule is sensitive to acute treatment with drugs acting at 5-HT_{1A} and 5-HT_{2A} receptors.

The 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) has been tested in several types of timing task, including the free-operant psychophysical procedure (Stubbs 1976) and the interval bisection task (Catania 1970) (for details of these timing schedules see Chapter 1). In the free-operant psychophysical procedure, 8-OH-DPAT displaced the psychometric function to the left, this being reflected in a reduction of the indifference point, T_{50} ; however, the slope of the function was only minimally affected by 8-OH-DPAT (Chiang et al. 2000b; Body et al. 2001, 2002b, 2004). Confirmation of the involvement of 5-HT_{1A} receptors in 8-OH-DPAT's effect was provided by the reversal of the effect by co-administration of the highly selective 5-HT_{1A} receptor antagonist *N*-[2-(4-[2-methoxyphenyl]-1-piperazinyl)ethyl]-*N*-2-pyridinylcyclohexanecarboxamide (WAY-100635) (Body et al. 2003, 2004).

8-OH-DPAT produced a very different pattern of effect in the interval bisection task. In this schedule, 8-OH-DPAT reduced the slope of the psychometric function, but did not alter T_{50} (Chiang et al. 2000b). In the discrete-trials psychophysical procedure (Body et al. 2002a), 8-OH-DPAT's effect on the function resembled that seen with the interval bisection task: the slope was reduced, but T_{50} was not altered (Body et al., 2002a).

The effects of the 5-HT_{2A/2C} receptor agonist 2,5,-dimethoxy-4-

iodoamphetamine (DOI) on timing performance are similar to those of 8-OH-DPAT. DOI reduced T_{50} in the free-operant psychophysical procedure, an effect that was reversed by the 5-HT_{2A} receptor antagonist ketanserin (Body et al. 2003, 2004), but reduced the slope of the function in the discrete-trials psychophysical procedure (see Section 4.3). Quipazine, an agonist with high affinity for both 5-HT₃ and 5-HT_{2A} receptors, also reduced T_{50} in the free-operant psychophysical procedure (see Section 3.3), and reduced the slope of the function in the discrete-trials psychophysical procedure (see Section 2.3). In both cases, quipazine's effect was reversed by ketanserin, implicating 5-HT_{2A} receptors in the effects of quipazine in both types of timing schedule.

Drug-induced displacement of the psychometric timing function is often interpreted in terms of a change in the period of the hypothetical pacemaker that is widely believed to underlie interval timing performance (Meck 1986, 1996; Gibbon et al. 1997). However, the divergent effects of the 5-HT_{1A} and 5-HT_{2A} receptor agonists on performance in different types of timing task defy a straightforward explanation in these terms, because according to classical pacemaker-based theories of timing, such as Scalar Expectancy Theory (Gibbon 1977) and the Behavioural Theory of Timing (Killeen and Fetterman 1988), the same pacemaker regulates timing performance on all voluntary timing tasks, and therefore it would be expected that a drug that affects pacemaker function would have at least qualitatively similar effects on performance on different types of timing schedule (see Zeiler 1998; Grondin 2001).

In searching for an alternative explanation for the effects of 5-HT_{1A} and 5-HT_{2A} receptor agonists on timing performance, it is appropriate to consider

procedural differences that might distinguish those tasks that reveal T_{50} -reducing effects of these agonists from those that do not (Ho et al. 2002). One possible distinguishing feature is suggested by Killeen et al.'s (1997) proposal that timing schedules can be classified according to the relation between the organism's behaviour and the interval being timed. According to Killeen et al.'s (1997) taxonomy, two major classes of timing schedule are *immediate* and *retrospective* timing schedules (Killeen and Fetterman 1988; Killeen et al. 1997). In immediate timing schedules the organism's behaviour comes under the control of time during an elapsing interval (*temporal differentiation*), whereas retrospective timing tasks require the organism to discriminate the durations of exteroceptive stimuli that have elapsed before the discriminative response is made (*temporal discrimination*) (see Section 1.3). The free-operant psychophysical procedure fulfils the criteria for an immediate timing schedule, whereas the interval bisection and discrete-trials psychophysical schedules belong to the category of retrospective timing tasks. Viewed in these terms, it is possible that acute 5-HT_{1A} and 5-HT_{2A} receptor stimulation results in a reduction of T_{50} only in immediate timing tasks. If this is the case, one might expect that agonists of these receptors would displace T_{50} in other immediate timing tasks.

The experiments reported here tested this prediction by examining the effects of 8-OH-DPAT and DOI on performance on the fixed-interval peak procedure. This schedule (Catania 1970; Roberts 1981) is one of the most widely used schedules in studies of interval timing in animals (see Hinton and Meck 1997; Matell and Meck 2004). In standard fixed-interval trials, reinforcement follows the first response after a fixed interval has elapsed; in

probe trials, reinforcement is omitted and responding is allowed to continue for a period several times longer than the fixed interval. Interval timing is revealed by the evolution of response rate during the course of the probe trials. Rising from a low level at the start of the trial, response rate attains a peak close to the designated time of reinforcer availability in the standard trials, and subsequently declines. The time of maximum response rate (peak time, t_{peak}) is the primary index of temporal differentiation, and has a theoretical status equivalent to that of T_{50} in the schedules described above (see Hinton and Meck 1997; Killeen et al. 1997). Like the free-operant psychophysical procedure, the fixed-interval peak procedure belongs to the category of immediate timing schedules (Killeen et al. 1997). However the two schedules differ in one important respect. In the former schedule, timing is measured from proportional choice between two concurrently available operanda, whereas the latter is a single-operandum schedule. Thus, while effects of drugs on T_{50} might be influenced by alterations of the propensity to switch from one operandum to the other (see Chiang et al. 1998), effects of drugs on t_{peak} cannot readily be accounted for by such a mechanism.

7.2. METHODS

7.2.1. Subjects

Thirty female Wistar rats aged approximately 4 months and weighing 250-290 g at the start of the experiment were used. Twelve rats were used for the first treatment series and eighteen for the second series (see below, *Drug*

treatment). The rats were housed individually under the same conditions as in Experiment 1 (see Section 2.2.1)

7.2.2. Apparatus

The rats were trained in operant conditioning chambers (Campden Instruments, Sileby, UK). Eighteen chambers were used; each rat was always tested in the same chamber. Twelve chambers were used for the first series of treatments (see below); these were equipped with motor-operated dippers which delivered a liquid reinforcer (50 μ l of a 0.6 M sucrose solution) (for description, see Section 2.2.2). The remaining six chambers were used for the second treatment series (see below); these were equipped with dispensers which delivered 45-mg food-pellet reinforcers (for description, see Section 4.2.2). Only one retractable lever was used in these experiments; this was the left-hand lever for nine rats and the right-hand lever for the other nine. The same computer as that used in Experiment 1 was used to control the schedule and record the behavioural data (see Section 2.2.2).

7.2.3 Behavioural training

At the start of the experiment, the food-deprivation regimen was started and the rats were gradually reduced to 80% of their free-feeding body weights. They were then trained to press the levers, and were exposed to a continuous reinforcement schedule for three daily sessions. Thereafter, the rats underwent 50-minute training sessions under the fixed-interval 30-s peak procedure as

described below, seven days a week, at the same time each day during the light phase of the daily cycle (between 8:00 and 13:00 hours). Each session consisted of 32 trials separated by 10-s intertrial intervals. Trials started with insertion of the lever into the chamber, and terminated with lever withdrawal. In fixed-interval trials (16 per session), reinforcement was delivered following the first response emitted after 30 s had elapsed since the onset of the trial. In probe trials (16 per session), reinforcement was omitted, and the lever remained in the chamber for 120 s. The fixed-interval and probe trials occurred in a pseudo-random sequence with the constraint that no more than three trials of either type occurred in succession. Timing behaviour was assessed from performance in the probe trials.

7.2.4. Drug treatment

The drug treatment regimen started after 90 sessions of preliminary training under the fixed-interval peak procedure. Treatments were given by subcutaneous injection (1.0 ml kg⁻¹ body weight). The protocol for subcutaneous injections was the same as experiment 1 (see Section 2.2.4.). In the first series ($n=12$) the treatments were 8-OH-DPAT HBr (0.05 mg kg⁻¹), WAY-100635 (0.1 mg kg⁻¹), and 8-OH-DPAT HBr (0.05 mg kg⁻¹) + WAY-100635 (0.1 mg kg⁻¹). In the second series ($n=18$) the treatments were (-)-DOI HCl (0.25 mg kg⁻¹), ketanserin tartrate (2 mg kg⁻¹), and (-)-DOI HCl (0.25 mg kg⁻¹) + ketanserin tartrate (2 mg kg⁻¹). Doses refer to weights of the salt.

7.2.5. Data analysis

Response rate was recorded in successive two-second epochs of the probe trials. For each rat, mean response rate R , for each treatment condition, derived from all the sessions in which that treatment was administered (see above), was plotted against time measured from the onset of the trial, t . The following modified Gaussian function was fitted to each rat's data (Buhusi 2005):

$$R = a \times e^{\left[-0.5 \times \left(\frac{t - t_{peak}}{b} \right)^2 \right]} + [c + d \times (t - t_{peak})] \quad [1]$$

where $(a+c)$ is the estimated peak response rate, t_{peak} is the peak time (location of the peak of the Gaussian component of the function), b represents the spread of the function (standard deviation of the Gaussian component); the right-hand term is a linear ramp of slope d and an ordinate value c at time $t=t_{peak}$. This function has been found to provide an acceptable description of performance in the peak procedure (Buhusi et al. 2005; MacDonald and Meck 2005). The following measures were derived for each rat under each treatment condition: the peak time (t_{peak}), the peak response rate $(a+c)$, and the Weber fraction (coefficient of variation of the Gaussian component of the function: b/t_{peak}). Goodness of fit of the fitted functions was expressed as r^2 . These measures were compared across treatments by repeated-measures analysis of variance. In the case of a significant effect of treatment, comparisons were made between each active treatment and the vehicle-alone condition using Dunnett's test, and between the agonist-alone and agonist+antagonist treatments, using Neuman-Keul's test (significance criterion, $P<0.05$).

7.3. RESULTS

7.3.1. 8-OH-DPAT

The group mean data obtained in the probe trials are shown in Figure 7.1. The left-hand panel shows the absolute response rates, and the right-hand panel the response rates expressed as a percentage of the maximum rate. Table 7.1 shows the group mean (\pm SEM) values of the timing parameters derived from fitting the modified Gaussian function to the data from the individual rats.

Peak time, t_{peak} . Under the vehicle-alone treatment condition, t_{peak} (32.5 ± 1.4 s) was close to the scheduled reinforcement time (30 s). Analysis of variance of the t_{peak} data revealed a significant effect of treatment [$F(3,33)=4.0$, $p<0.02$]. Multiple comparisons indicated that 8-OH-DPAT (0.05 mg kg^{-1}) significantly reduced t_{peak} . WAY-100635 (0.1 mg kg^{-1}), administered alone, had no significant effect on t_{peak} ; however it significantly antagonized the reduction of t_{peak} induced by 8-OH-DPAT. The value of t_{peak} seen following combined treatment with 8-OH-DPAT + WAY-100635 did not differ significantly from that seen following vehicle-alone treatment.

Weber fraction. There was no significant overall effect of treatment on the Weber fraction [$F(3,33)=2.1$, $p>0.1$].

Peak response rate. There was a significant overall effect of treatment on peak response rate [$F(3,33)=2.9$, $p<0.05$]. Multiple comparisons showed that all three active treatments (8-OH-DPAT, WAY-100635 and 8-OH-DPAT + WAY-100635) produced a significant reduction of peak response rate compared to the vehicle-alone treatment.

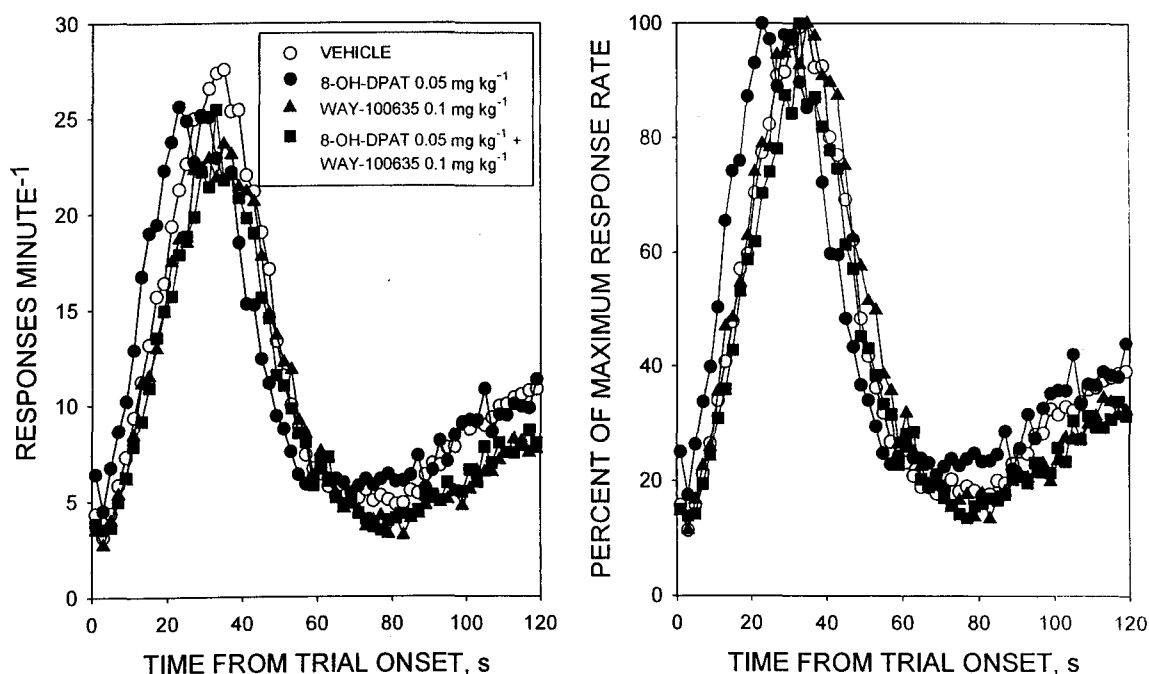


Figure 7.1. Effects of 8-OH-DPAT (0.05 mg kg^{-1}), WAY-100635 (0.1 mg kg^{-1}), and combined treatment with 8-OH-DPAT (0.05 mg kg^{-1}) + WAY-100635 (0.1 mg kg^{-1}) on performance on peak fixed-interval 30-s schedule. *Left-hand panel.* Ordinate: absolute response rate (responses minute⁻¹); abscissa: time from trial onset (s). Points are group mean data from successive 2-s time bins: open circles, vehicle treatment; filled circles, 8-OH-DPAT; filled triangles, WAY-100635; filled squares, 8-OH-DPAT + WAY-00635. *Right-hand panel.* Ordinate: response rate expressed as percent of maximum response rate (other conventions as in left hand panel). Note the leftward displacement of the peak function (reduction of peak time) induced by 8-OH-DPAT, and reversal of this effect by WAY-100635.

Table 1. Effects of 8-OH-DPAT, WAY-100635 and combined treatment with 8-OH-DPAT + WAY-100635 on performance on the fixed-interval peak procedure

Parameters derived from fit of modified Gaussian function (mean \pm SEM)				
Treatment	t_{peak} , s	Weber fraction	r^2	Peak response rate (responses min ⁻¹)
Vehicle	32.5 \pm 1.4	0.43 \pm 0.04	0.932 \pm 0.016	28.6 \pm 4.6
8-OH-DPAT 0.5 mg kg ⁻¹	26.5 \pm 1.7 *	0.61 \pm 0.07	0.800 \pm 0.051	23.7 \pm 6.2 *
WAY-100635 0.1 mg kg ⁻¹	30.6 \pm 1.6	0.74 \pm 0.18	0.789 \pm 0.057	23.5 \pm 5.3 *
8-OH-DPAT 0.5 mg kg ⁻¹ + WAY-100635 0.1 mg kg ⁻¹	32.4 \pm 1.4 #	0.40 \pm 0.06	0.794 \pm 0.094	23.2 \pm 4.5 *

Significance of difference from vehicle condition, * $p < 0.05$; significance of difference from 8-OH-DPAT 0.5 mg kg⁻¹, # $p < 0.05$

7.3.2. DOI

The group mean data obtained in the probe trials are shown in Figure 7.2. The left-hand panel shows the absolute response rates, and the right-hand panel the response rates expressed as a percentage of the maximum rate. Table 7.2 shows the group mean (\pm SEM) values of the timing parameters derived from fitting the modified Gaussian function to the data from the individual rats.

Peak time, t_{peak} . Under the vehicle-alone treatment condition, t_{peak} (33.2 ± 1.3 s) was close to the scheduled reinforcement time (30 s). Analysis of variance of the t_{peak} data revealed a significant effect of treatment [$F(3,51)=6.0$, $p<0.01$]. Multiple comparisons indicated that DOI (0.25 mg kg^{-1}) significantly reduced t_{peak} . Ketanserin (2 mg kg^{-1}), administered alone, had no significant effect on t_{peak} ; however it antagonized the reduction of t_{peak} induced by DOI. The value of t_{peak} seen following combined treatment with DOI + ketanserin did not differ significantly from that seen following vehicle-alone treatment.

Weber fraction. The overall effect of treatment on the Weber fraction fell just short of statistical significance [$F(3,51)=2.7$, $p=0.055$]. The values obtained following treatment with DOI and ketanserin were somewhat higher than that seen following vehicle-alone treatment.

Peak response rate. There was a significant overall effect of treatment on peak response rate [$F(3,51)=2.9$, $p<0.05$]. Multiple comparisons showed that DOI produced a significant reduction of peak response rate compared to the vehicle-alone treatment.

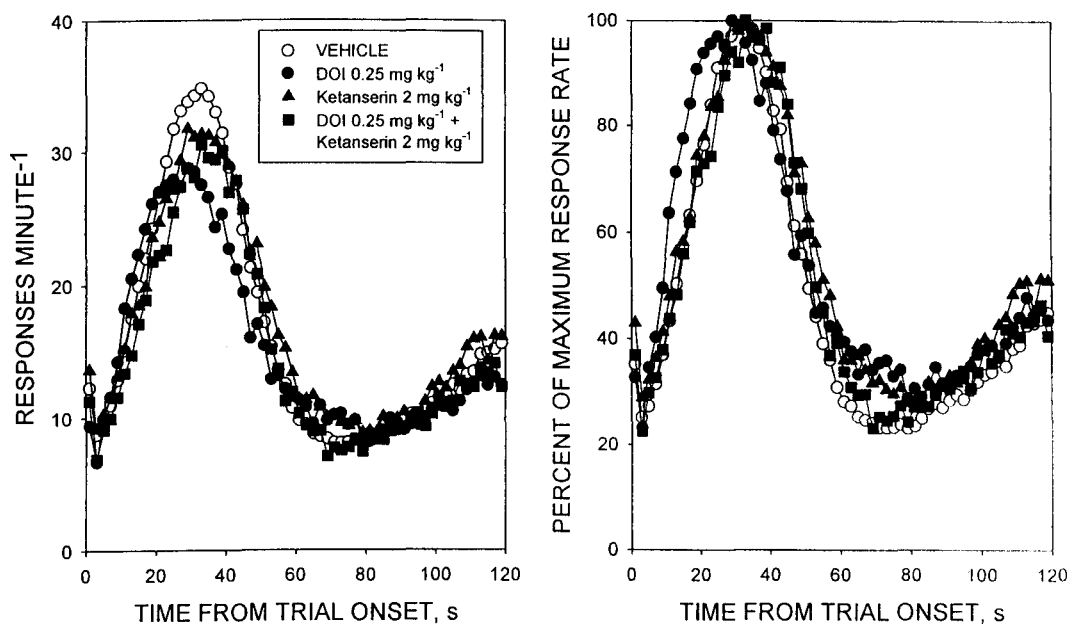


Figure 7.2. Effects of DOI (0.25 mg kg⁻¹), ketanserin (2 mg kg⁻¹), and combined treatment with DOI (0.25 mg kg⁻¹) + ketanserin (2 mg kg⁻¹) on performance on the peak fixed-interval 30-s schedule. *Open circles*, vehicle; *filled circles* DOI; *filled triangles*, ketanserin; *filled squares*, DOI + ketanserin. Other conventions as in Figure 1. Note the leftward displacement of peak function (reduction of peak time) induced by DOI, and reversal of this effect by ketanserin.

Table 2. Effects of the DOI, ketanserin and combined treatment with DOI + ketanserin on performance on the fixed-interval peak procedure

Parameters derived from fit of modified Gaussian function (mean ± SEM)				
Treatment	t_{peak} , s	Weber fraction	r^2	Peak response rate (responses min ⁻¹)
Vehicle	33.2 ± 1.3	0.45 ± 0.04	0.801 ± 0.032	36.0 ± 6.2
DOI 0.25 mg kg ⁻¹	29.7 ± 1.1 *	0.60 ± 0.04	0.797 ± 0.040	29.7 ± 5.0 *
ketanserin 2 mg kg ⁻¹	33.6 ± 1.2	0.61 ± 0.09	0.797 ± 0.040	32.5 ± 5.6
DOI 0.25 mg kg ⁻¹ + ketanserin 2 mg kg ⁻¹	34.5 ± 1.3 #	0.45 ± 0.04	0.804 ± 0.036	30.9 ± 5.3

Significance of difference from vehicle condition, * $p<0.05$; significance of difference from DOI 0.25 mg kg⁻¹, # $p<0.05$

7.4. DISCUSSION

Performance in the fixed-interval peak procedure seen in these experiments conformed to the characteristic bell-shaped response-rate function reported in many previous experiments (see Hinton and Meck 1997). Performance could be described by a modified Gaussian curve ('Gaussian plus ramp' function: Buhusi et al. 2005), enabling estimates of t_{peak} and the Weber fraction to be derived from each rat under each treatment condition.

The 5-HT_{1A} receptor agonist 8-OH-DPAT displaced the response-rate function to the left. This was reflected in a significant reduction of t_{peak} . The 5-HT_{1A} receptor antagonist WAY-100635 had no significant effect on t_{peak} ; however, it completely abolished the reduction of t_{peak} produced by 8-OH-DPAT. 8-OH-DPAT is a potent agonist of 5-HT_{1A} receptors; however, it also has some partial agonist activity at 5-HT₇ receptors (see Thomas and Hagan 2004). The ability of WAY-100635 completely to antagonize 8-OH-DPAT's effect on t_{peak} strongly implicates 5-HT_{1A} receptors in this effect of 8-OH-DPAT, since WAY-100635 is highly selective for the 5-HT_{1A} site (see Hoyer et al. 2002; Lanfumey and Hamon 2004).

8-OH-DPAT's ability to reduce t_{peak} is consistent with its ability to reduce the indifference time, T_{50} , in another immediate timing schedule, the free-operant psychophysical procedure (Chiang et al. 2000; Body et al. 2001, 2002b, 2004). 8-OH-DPAT's effect on T_{50} is also sensitive to antagonism by WAY-100635, suggesting that the same receptor population may be responsible for both effects. 5-HT_{1A} receptors occur both on cell bodies and dendrites of 5-HTergic neurones in the raphe nuclei (somatodendritic

autoreceptors) and on postsynaptic membranes in the forebrain target regions of the 5-HTergic projection (see Lanfumey and Hamon 2004). It remains uncertain whether the effects of 8-OH-DPAT seen here were mediated by presynaptic or by postsynaptic 5-HT_{1A} receptors. However, circumstantial evidence favours postsynaptic receptors, because 8-OH-DPAT's effect on performance on the free-operant psychophysical procedure is impervious to destruction of the ascending 5-HTergic pathways (Body et al. 2001, 2002b, 2004).

The 5-HT_{2A/2C} receptor agonist DOI also reduced t_{peak} . Ketanserin, when administered alone, had no significant effect on t_{peak} ; however, it completely antagonized the effect of DOI on this parameter. It is likely that the 5-HT_{2A} receptor subtype is likely to have been responsible for mediating DOI's effect on t_{peak} in this experiment. Although DOI has approximately equivalent affinity for 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors, ketanserin has an 80-100 times higher affinity for the 5-HT_{2A} receptor than for the other two 5-HT₂ receptor subtypes (Baxter et al. 1995; Barnes and Sharp 1999); moreover, 5-HT_{2B} receptors are very sparsely expressed in the central nervous system (Barnes and Sharp 1999). Confirmation of this suggestion will require further experiments using more selective 5-HT_{2A} and 5-HT_{2C} receptor antagonists.

As with 8-OH-DPAT, so also with DOI, the present finding with the fixed-interval peak procedure has its counterpart in the free-operant psychophysical procedure. DOI produced a dose-dependent reduction of T_{50} in this schedule, which could be completely reversed by ketanserin, suggesting mediation of the effect by 5-HT_{2A} receptors (Body et al. 2002b, 2004; see also Experiment 5). Thus the present results, taken together with previous findings

with the free-operant psychophysical procedure, suggest that 5-HT_{1A} and 5-HT_{2A} receptors mediate qualitatively similar effects on temporal differentiation.

As well as reducing t_{peak} , both 8-OH-DPAT and DOI also induced some broadening of the peak function, this being reflected in an increase of the Weber fraction (marginally statistically significant only in the case of DOI), and a modest reduction of the peak response rate. However, the effects of 8-OH-DPAT and DOI on the peak response rate were not reversed by their respective antagonists, WAY-100635 and ketanserin, suggesting that they may constitute non-specific effects on performance.

The reduction of the indices of central tendency of timing in the immediate timing tasks (t_{peak} in the fixed-interval peak procedure and T_{50} in the free-operant psychophysical procedure) produced by 8-OH-DPAT and DOI stands in contrast to the effects of these drugs on the analogous measures derived from performance on retrospective timing tasks. As discussed above, 5-HT_{1A} and 5-HT_{2A} receptor agonists either have no effect on T_{50} in these tasks, or in some cases may even increase this parameter (see Chapter 2). This dissociation of effects on temporal differentiation and temporal discrimination is not easy to reconcile with an interaction of the drugs in question with the ubiquitous internal clock that is purported to underlie interval timing in all its guises (Gibbon 1977; Killeen and Fetterman 1988). The impasse could be breached by postulating two timekeepers, subserved by different neuropharmacological mechanisms, with separate responsibilities for temporal differentiation and temporal discrimination (see Ho et al. 2002). However, such an unparsimonious approach may be unwarranted at this stage, in the light of

evidence that a number of other neurobiological interventions do produce coherent effects on the two forms of interval timing (Gibbon et al. 1997; Hinton and Meck 1997; Matell and Meck 2004).

An alternative tactic may be to search for other ('non-timing') behavioural processes that are differentially represented in immediate and retrospective timing schedules, and which may differ in their sensitivities to 5-HT_{1A} and 5-HT_{2A} receptor stimulation. One candidate process discussed above, the propensity for switching between concurrently available operanda, which is known to be sensitive to manipulation of 5-HTergic function (Al-Ruwaitea et al. 1997, 1999; Chiang et al. 1999), is rendered somewhat unlikely by the present findings, since the fixed-interval peak procedure used in this experiment employed only a single operandum (however, see Ho et al. 1998, for discussion of the possible involvement of 'switching' in performance on the fixed-interval peak procedure). Another candidate process may be 'attention-sharing' (Meck and Benson 2002; Buhusi and Meck 2002; Buhusi 2003). It has been suggested that dopaminergic manipulations may influence timing by interacting both with the hypothetical pacemaker and with attentional processes, and that inconsistencies between effects of dopamine receptor agonists and antagonists on different types of timing task may reflect differential interaction with these two processes (Buhusi 2003). Further work will be needed to establish whether such an explanation can help to account for the divergent effects of 5-HT_{1A} and 5-HT_{2A} receptor stimulation on performance on immediate and retrospective timing tasks. One approach to addressing this question could be to examine the effects of the agonists on timing performance in the two types of timing task using different time ranges.

For example, it has been proposed that effects on the function of the hypothetical clock should be reflected in a shift of the psychometric function whose magnitude is proportional to the criterion duration, whereas effects on attentional processes should be reflected in a shift of the function which is uniform across different criterion durations (Hinton and Meck 1997; Buhusi 2003).

The neuronal mechanisms whereby 8-OH-DPAT and DOI exert their similar effects on temporal differentiation in the fixed-interval peak procedure remain to be elucidated. 5-HT_{1A} receptors are expressed both on post-synaptic cells and on the cell bodies and dendrites of 5-HTergic neurones; as discussed above, it is likely that the effect of 8-OH-DPAT seen here were mediated by post-synaptic receptors. 5-HT_{2A} receptors are located almost exclusively on postsynaptic membranes (Barnes and Sharp 1999; Hoyer et al. 2002). However, it is unlikely that the 5-HT_{1A} and 5-HT_{2A} receptors responsible for the similar effects of 8-OH-DPAT and DOI on temporal differentiation are located on the same group of neurones, because these two receptor subtypes generally mediate opposite effects on neuronal excitability (hyperpolarization and depolarization, respectively: Barnes and Sharp 1999; Hoyer et al. 2002; Lanfumey and Hamon 2004; Leysen 2004). Further experiments employing direct injection of the agonists into discrete brain regions may help to reveal the neuroanatomical location of the two receptor populations.

CHAPTER 8

GENERAL DISCUSSION

8.1. The role of 5-HT receptors in timing

The results of this project confirm and extend the previous findings on the effects of 5-HT receptor stimulation on temporal discrimination and temporal differentiation.

In experiment 1 it was found that the non-selective 5-HT₂/5-HT₃ receptor agonist quipazine disrupted temporal discrimination in the discrete-trials psychophysical procedure. This effect of quipazine was probably mediated by 5-HT₂ rather than 5-HT₃ receptors, because it was not blocked by the selective 5-HT₃ receptor antagonist MDL-72222, whereas it was completely reversed by the 5-HT₂ ('5-HT_{2A}-preferring') antagonist ketanserin. Experiment 3 extended these findings by showing that the 5-HT_{2A/2C} receptor agonist DOI disrupted temporal discrimination in a similar manner to quipazine, and that DOI's effect could be antagonized by the highly selective 5-HT_{2A} receptor antagonist MDL-100907.

In a previous experiment, Body et al. (2002a) found that 5-HT_{1A} receptor agonist 8-OH-DPAT also impaired temporal discrimination in the discrete-trials psychophysical procedure. The findings in experiments 1 and 3, taken together with Body et al.'s (2002a) results, suggest that 5-HT_{1A} and 5-HT_{2A} receptors mediate qualitatively similar effects on temporal discrimination. The effect of 8-OH-DPAT described by Body et al. (2002a) was evidently mediated by a postsynaptic receptor population, since the effect survived destruction of ascending 5-HTergic pathways by intra-raphe injection of 5,7-DHT. It is likely that the 5-HT_{2A}-mediated effect seen here was also mediated by a postsynaptic receptor population, because 5-HT_{2A} receptors have

been found mainly on postsynaptic membranes (see section 1.2.3.3.). Whether or not the two receptor subtypes reside on the same population of neurones is a question which will need to be addressed in future experiments.

The disruptive effect of 5-HT_{2A} receptor stimulation (and 5-HT_{1A} receptor stimulation: Body et al. 2002a) consisted of a marked degradation of stimulus control, as revealed by an increase in the Weber fraction. There was also a tendency for the indifference point, T_{50} , to be increased. Increases in T_{50} can be interpreted in more than one way. One of the possible explanations is that DOI and quipazine might have increased the period of the hypothetical pacemaker (Gibbon 1991; Hinton and Meck 1997; see section 8.3 for further discussion).

Quipazine (experiment 2) and DOI (experiment 5) also affected temporal differentiation in the free-operant psychophysical procedure. These effects appear to be mediated by 5-HT_{2A} receptors, since they were reversed by ketanserin and MDL-100907, respectively. These effects resembled the effects of DOI on performance on this schedule previously reported by Body et al. (2003, 2004). Body et al. (2004) confirmed the postsynaptic location of the receptors responsible for DOI's effect on temporal differentiation, by demonstrating that the effect of DOI was not reduced after destruction of the ascending 5-HTergic pathways.

Although 5-HT_{2A} receptors mediate effects on both temporal discrimination and temporal differentiation, the effects on the two types of timing performance are strikingly different. In contrast to the increase in the Weber fraction and T_{50} seen in the former case, T_{50} was consistently reduced by 5-HT₂ receptor stimulation in the case of temporal differentiation (experiments

2 and 5). This is in agreement with earlier findings (Body et al. 2002b, 2003, 2004). The theoretical implications of this discrepancy is discussed in section 8.3.

The anatomical location of the 5-HT_{2A} receptors responsible for the effects on temporal discrimination and differentiation remains unknown. Evidence from experiments 4 and 6 suggests that they are unlikely to reside in the dorsal striatum. 5-HT_{2A} receptors have been found in many parts of the central nervous system, and a considerable amount of work may be needed to track down the relevant receptor population. In view of the theoretical importance of the qualitative discrepancy between effects on temporal discrimination and temporal differentiation (see below), it will be of considerable interest to discover whether the same or different populations of 5-HT_{2A} receptors mediate these effects.

Experiment 7 examined the effects of DOI and 8-OH-DPAT on temporal differentiation on a different type of immediate timing schedule, the fixed-interval peak procedure. Both drugs displaced the peak time, t_{peak} , to the left, an effect that is qualitatively similar to the reduction of T_{50} seen in the free-operant psychophysical procedure. These results thus extend Body et al.'s (2004) findings that 5-HT_{1A} and 5-HT_{2A} receptor stimulation have similar effects on temporal differentiation.

8.2. 5-HT and Dopamine

The interaction between 5-HT and dopamine in the brain is not a simple one, as reviewed in Chapter 1. 5-HT plays a complicated set of roles in the brain that

are difficult to encompass with a single theory. In some cases, dopaminergic function has been found to be antagonized by 5-HT receptor stimulation; however, in other cases the two monoamines appear to have synergistic effects. The situation is further complicated by the fact that different 5-HT receptor subtypes have been found to mediate opposing effects on dopaminergic function in some cases (see Daw et al. 2002). In the striatum, endogenous 5-HT has no influence on dopamine release under basal conditions, but positively modulates dopamine outflow when nigro-striatal dopaminergic transmission is activated (Lucas et al. 2000). In the nucleus accumbens selective blockade of 5-HT_{2C/2B} receptor subtypes increases dopamine release (Di Matteo et al. 1998).

According to some pacemaker-based theories of interval timing, it has been suggested that the facilitatory effect of striatal dopaminergic mechanisms is opposed by an inhibitory effect of 5-HT on the hypothetical pacemaker (Hinton and Meck 1997). The results of this project, taken in conjunction with earlier findings, suggest that this proposal may not be correct. Thus, numerous studies have reported that t_{peak} in the peak procedure is reduced by the dopamine-releasing agent amphetamine, and increased by dopamine D₂ receptor antagonists (see Meck 1986, 1996; Gibbon et al. 1997; Matell and Meck 2000). The results of experiment 7 indicate that both 5-HT_{1A} and 5-HT_{2A} receptor stimulation can also reduce t_{peak} . Moreover, the indifference point, T_{50} , in another temporal differentiation schedule, the free-operant psychophysical procedure, is consistently reduced by amphetamine (Chiang et al. 2000a) and by 5-HT_{1A} (Body et al., 2001, 2002b, 2004) and 5-HT_{2A} (Body et al. 2003, 2004; present project, experiments 2 and 5) receptor agonists.

There is good evidence that 5-HT₃ receptors contribute to the regulation of dopamine release (Blandina et al. 1989; Carboni et al. 1989; Zazpe et al. 1994; Cervo et al. 1996). It might therefore be expected that 5-HT₃ receptor stimulation would alter timing performance, an expectation that found no support in experiment 2. The explanation for this discrepancy may lie in the regional differences in the nature of 5-HT/dopamine interactions. There is evidence that dopamine releasing effects of 5-HT₃ receptors is mainly restricted to the structures innervated by mesocortical and mesolimbic dopaminergic projections, rather than the dorsal striatum, which receives its input from the nigrostriatal pathway (Wang et al. 1996; De Deurwaerdere et al. 1998; Porras et al. 2003). The failure of 5-HT₃ receptor stimulation to affect temporal differentiation in experiment 2 may therefore reflect a primary involvement of dorsal rather than the ventral striatal dopaminergic mechanisms in this behaviour (Hinton and Meck 1997; Matell and Meck 2000).

8.3. Theoretical Implications

One of the most consistent findings that can be extracted from these experiments is that temporal discrimination and temporal differentiation can be affected in qualitatively different ways by the same 5-HT receptor agonists. This finding cannot easily be reconciled with the concept of a single time-keeper mechanism that is believed to underlie all types of interval timing behaviour (see Hinton and Meck 1997; Matell and Meck 2000; Meck 2005). As succinctly stated by Zeiler, “any model that posits a unitary internal clock, would seem to imply that an intervention that alters its speed, should produce

qualitatively similar disruptions of temporal differentiation and temporal discrimination” (Zeiler 1998).

The present project has provided evidence that 5-HT_{2A} receptor stimulation reduces T_{50} in a temporal differentiation schedule and increases it in a temporal discrimination schedule. According to pacemaker-based theories of timing, this would seem to imply that the pacemaker was speeded up in the former case and slowed down in the latter. The present results join an increasing body of findings suggesting that stimulation of the same type of receptor in different timing tasks can produce different results. For example, Chiang et al. (2000b) found that the 5-HT_{1A} receptor agonist 8-OH-DPAT increased the Weber fraction in the interval bisection task, without affecting the bisection point; and Body et al. (2001a) found a similar effect of 8-OH-DPAT in another retrospective timing schedule, the discrete-trials psychophysical procedure. In contrast, 8-OH-DPAT selectively reduced T_{50} in the free-operant psychophysical procedure (Chiang et al. 2000b; Body et al. 2003, 2004). Thus 8-OH-DPAT has qualitatively different effects in immediate and retrospective timing schedules.

The difficulty posed for pacemaker-based theories of timing by results such as these has been discussed by Chiang et al. (2000a). One of two courses of action seems to be unavoidable. Either the notion of a single pacemaker underlying both temporal discrimination and temporal differentiation has to be abandoned, or the effects of drugs on one type (or even both types) of timing task must be attributed to some ‘non-pacemaker-based’ action. A difficulty with the latter alternative is that drug-induced changes in pacemaker function have traditionally been inferred from changes in the locus of T_{50} derived from

psychometric functions. If a change in the locus of T_{50} can no longer be relied upon as evidence for a change in pacemaker function, the testability of pacemaker-based theories is considerably compromised. It may be noted that the pacemaker concept is still essentially a 'hypothetical construct', and attempts to link it to any particular neural structures remain highly speculative. It is to be hoped that improved understanding of the neural mechanisms of timing will eventually help to resolve this controversy (see Buhusi 2003; Matell and Meck 2000, 2004).

8.4. **Future research**

The results of the present project have identified 5-HT_{1A} and 5-HT_{2A} receptors as mediators of substantive effects on interval timing behaviour. An obvious area for continued research is the exploration of the roles of other 5-HT receptor subtypes. This is especially relevant in the case of those receptors that are known to contribute to the regulation of dopamine release in the component structures of cortico-striato-thalamo-cortical loops, which are the current focus of theorizing about the neural basis of timing behaviour (Buhusi 2003; Matell and Meck 2004; Meck 2005).

It will also be important to explore the anatomical location of the 5-HT receptor populations that are involved in timing. Experiments 4, 5 and 6 constitute an initial move in this direction; it seems that the 5-HT_{2A} receptors responsible for effects on temporal discrimination and temporal differentiation are probably not located in the dorsal striatum. However, as discussed in chapter 6, 5-HT_{2A} receptors are expressed in more than one component of the

cortico-striato-thalamo-cortical loops (Pompeiano et al. 1994; Wright et al. 1995; Hamada et al. 1998; Cornea-Herbert et al. 1999; Bubser et al. 2001; Hoyer et al. 2002); additionally there is evidence that 5-HT_{2A} receptors are present on dopaminergic neurones of the ventral tegmental area and substantia nigra. Whether or not these receptor populations are responsible for the effects of 5-HT_{2A} receptor agonists on interval timing behaviour is an open question that awaits further investigation.

Finally, future experiments should address the possibility that sex differences may exist in the quantitative features of interval timing behaviour, and in the sensitivity of timing behaviour to drugs acting at 5-HT receptors. All the experiments described in this thesis were carried out on female Wistar rats. This facilitates comparison of the present results with previous results obtained in this laboratory, which also employed female rats. It is unlikely that behavioural rhythms associated with the oestrus cycle would have had any systematic effect on the performances seen in the present experiments, because each treatment was administered to each rat five times at 3- or 4-day intervals, the order of treatments being counterbalanced across rats. Thus confounding of the treatment conditions with oestrus status is unlikely to have occurred. Nevertheless, the possibility that male rats might have responded differently from female rats to the treatments used in these experiments deserves serious consideration in view of recent evidence for oestrogen-induced changes in neurotransmitter metabolism and synaptic plasticity in the rat (Bi et al. 2001; McEwen et al. 2001).

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APPENDIX

Chemical names of the drugs identified in the text by pharmaceutical company code numbers

8-OH-DPAT	8-hydroxy-2-(di-n-propilamino)tetraline
BW-723-C86	1-[5(2-thienylmethoxy)-1H-3-indolyl]propan-2-amine hydrochloride
CP-93129	5H-Pyrrolo[3,2-b]pyridine-5-one, 1,4-dihydro-3-(1,2,3,6- tetrahydro-4-pyridinyl)
CP-94253	(5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-H-p[3,2- b]pyridine hydrochloride
DOI	2,5-Dimethoxy-4-iodoamphetamine hydrochloride
DR-4004	2 α -(4-(4-phenyl-1,2,3,6-tetrahydropyridyl)butyl)-2 α ,3,4,5- tetrahydrobenzo[cd]indol-2-(1H)-one
GR-46611	2-propenamide, 3-[3-[2-(dimethylamino)ethyl]-1H-indol-5-yl]- N-[(4-methoxyphenyl)methyl]
GR-55562	3-[3-(dimethylamino)propyl]-4-hydroxy-N-[4-(4- pyridinyl)phenyl]benzamide
GR-113808	[1-2[(methylsulfonyl)amino)ethyl]-4-piperidinyl]methyl-1- methyl-1H-indole-3-carboxilate
GR-125487	[1-[2-(methylsulfonyl)amino]ethyl]-4-piperidinyl]-methyl 5- fluoro-2-methoxy-1H-indole-3-carboxilate
GR-127935	N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2C-methyl- 4C-(5-methyl)-1,2,4-oxadiazol-3-yl)-1,1C-biphenyl-4- carboxamide hydrochloride
LY-334370	5-(4-fluorobenzoyl)-amino-3-(1-methylpiperidin-4-yl)-1H-indole fumarate
LY-344864	N-[(3R)-3-(dimethylamino)-2,3,4,9-tetrahydro-1H-carbazol-6- yl]-4-fluoro-benzamide
m-CPBG	1-(m-chlorophenyl)biguanide
m-CPP	1-(3-chlorophenyl)piperazine

MDL-72222	3-Tropanyl-3,5-dichlorobenzoate
MDL-100907	(±)2,3-dimethoxyphenyl-1-[2-(4-piperidine)-methanol]
PNU-109291	(S)-3,4-dihydro-1-[2-[4-(4-methoxyphenyl)-1-piperazinyl]ethyl]-N-methyl-1H-2-benzopyran-6-carboxamide.
Ro-04-6790	4-amino-N-[2,6-bis(methylamino-4-pyrimidinyl)-benzensulfonamide
Ro-60-0175	(S)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamineRS-67333: (1-(4-amino-5-chloro-2-methoxyphenyl)-3-(1-n-butyl-4-piperidinyl)-1-propanone
RS-67506	(1-(4-amino-5-chloro-2-methoxyphenyl)-3-[1-[2-[(methylsulfonyl)amino]ethyl]-4-piperidinyl]-1-propanone)
RS-67532	[1-(4-amino-5-chloro-2-(3,5-dimethoxy benzyloxyphenyl)-5-(1-piperidinyl)-1-pentanone]
RS-102221	8-[5-(5-amino 2,4-dimethoxyphenyl) 5-oxo-pentyl]-1,3,8-triazaspiro[4,5]decane-2,4-dione
RU-24969	1H-Indol, 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-butanedioate
SB-200464	N-(1-methyl-5-indonyl)-N'-(3-pyridyl) urea hydrochloride
SB-216641	[1,1'-Biphenyl]-4-carboxamide, N-[3-[2-(dimethylamino)ethoxy]-4-methoxyphenyl]-2'-m(5-methyl-1,2,4-oxadiazol-3-yl)
SB-224289	1'-methyl-5[[2'-methyl-4'-)5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]carbonyl-2,3,6,7-tetrahydrospiro[furo[2,3-f]indol-3,4'-piperidine]oxalate
SB-242084	6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl]indoline
SB-258719	(R)-3,N-dimethyl-N-[1-methyl-3-(4-methylpiperidin-1-yl)propyl]benzene sulphonamide
SB-271046	5-chloro-N-(4-methoxy-3-piperazin-1-yl-phenyl)-3-methyl-2-benzothiophenesulphonamide
WAY-100635	N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridyl)-cyclohexanecarboxamide trichloride